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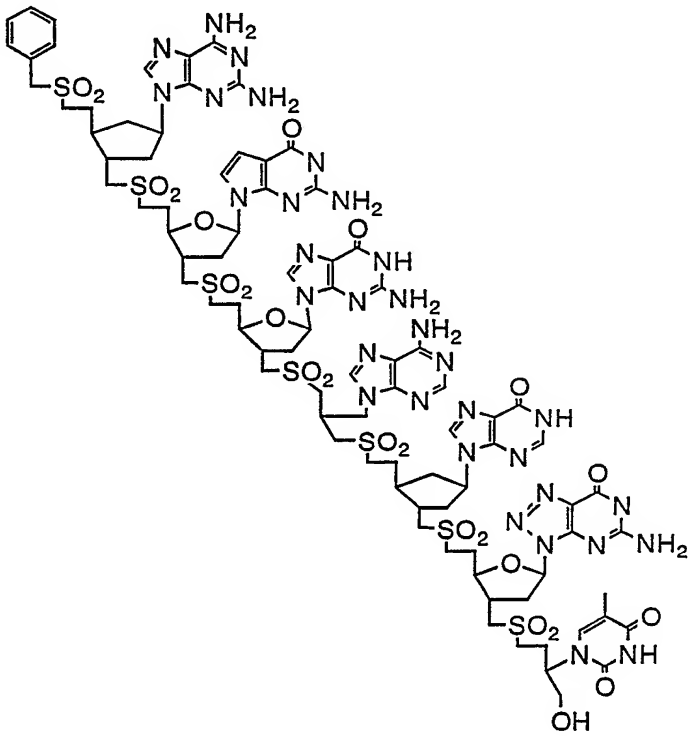
(54) Title: OLIGONUCLEOTIDE ANALOGS CONTAINING SULFUR

(57) Abstract

Analogs of DNA containing sulfides, sulfoxides, and sulfones as linking groups between subunits capable of forming bonds with natural oligonucleotides. The analogs are lipophilic, stable to chemical degradation under a wide range of conditions and stable to enzymatic degradation *in vivo*.

EXAMPLE OF OLIGONUCLEOTIDE ANALOG BINDING TO A-C-C-T-C-C-T



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OLIGONUCLEOTIDE ANALOGS CONTAINING SULFUR

Description

Introduction

As oligomers composed of five subunits (adenosine, A, cytidine, C, guanosine, G, uridine, U, and thymidine, T) joined by phosphodiester linkages, naturally occurring nucleic acids possess two notable properties. First, oligonucleotides bind to complementary oligonucleotides (where "complementarity rules" pair A in one oligonucleotide strand with either U or T in the other, and G in one oligonucleotide strand with C in the other, with the two strands anti-parallel). Second, they contain information in the sequences of bases in the oligonucleotide strand. Oligonucleotides serve as genes, genetic regulatory agents, intracellular messages, especially for the synthesis of proteins, and as intercellular messages.\(1)

Analogs of oligonucleotides are valuable, especially if they bind sequence-specifically to natural oligonucleotides and are stable under physiological conditions. For example, oligonucleotide analogs can serve as probes for complementary DNA. If they bind to oligonucleotides in vivo and disrupt their biological function (e.g., inhibit the synthesis of specific proteins), they can also serve as research tools and pharmaceuticals.

Natural oligonucleotides now serve these roles in special cases. For example, "anti-sense" RNA and DNA inhibit the expression of proteins.\(2) However, natural oligonucleotides are enzymatically degraded,\(3) and do not easily cross biological barriers to reach their "target" oligonucleotide. Thus, one application of natural oligonucleotides as research tools requires

that they be injected into individual cells.(2)

The prior art includes several non-polar polymeric analogs of oligonucleotides lacking phosphate groups to circumvent these problems, each not fully satisfactory for general use. Random hydrocarbon polymers bearing nucleoside bases cannot be synthesized with a defined structure.\(4) Oligonucleotides analogues with the phosphate replaced by a carboxyl group slowly hydrolyze upon standing at neutral pH.\(5) Carbamate analogs of oligonucleotides have geometrical constraints imposed by the linking group.\(6) Oligonucleotides containing 1,3 propanediol units between normal phosphate bases are not isosteric and cannot be prepared in the modified structural forms needed to modulate their binding affinity for natural oligonucleotides (vide infra).\(7) Finally, oligonucleotide analogs containing secondary hydroxyl groups and a bridging unit containing a thioether linkage have been proposed.\(8) This structure again cannot be prepared in the modified structural forms needed to modulate their binding affinity for natural oligonucleotides (vide infra). Further, it appears that to form standard double helical structures with natural oligonucleotides, these molecules must adopt an unfavorable conformation where a polar hydroxyl group is adjacent to a non-polar polymethylene unit.

Isosteric analogs of DNA where one oxygen of the phosphate group is replaced by a methyl group have been prepared. These "methylphosphonates" show interesting biological properties.\(9) They appear to cross cell membranes,\(10) and one isomer appears to form double helical structures with complementary natural

oligonucleotides, and block their biological action.

However, oligomethylphosphonates are complex mixtures of isomers, and only one part of the mixture is biologically active.\(11) Further, methylphosphonates are unstable to alkaline conditions required for their synthesis. Finally, these analogs again are not apparently accesible in the modified structural forms needed to modulate their binding affinity for natural oligonucleotides.

Invention

This invention (Figure 1) provides oligonucleotide analogs and building blocks for synthesizing these analogs, that can be designed to bind specifically to target oligonucleotides, and that have the following properties:

(a) They present an ordered array of groups capable of forming hydrogen bonds to a natural oligonucleotide, and thus bind to natural oligonucleotides.

(b) Their affinity for natural oligonucleotides can be modulated (i.e., strengthened or weakened) by incorporating different subunits with different intrinsic binding abilities.

(c) They are stable under a range of biological and chemical conditions.

(d) They do not contain groups that bear a charge at neutral pH, and their overall polarity is modulatable by varying the choice of building blocks.

These analogs contain a sulfur linkage (thioether, sulfoxide, or sulfone) instead of phosphate to join oligonucleotide analog building blocks.

Experiments in my laboratory with Escherichia coli showed

that the biological effects of organic sulfides (compounds with the general formula RSR') sulfoxides (with the general formula RSOR') and sulfones (with the general formula RSO_2R') could be explained by assuming that molecules with these structural units could penetrate biological barriers. Further, dimethyl sulfone and sulfolane were found to assist natural oligonucleotides in crossing cell membranes. Also, sulfides, sulfoxides, and sulfones proved to be stable to chemical and biochemical degradation. Finally, sulfones are achiral isosteric and isoelectronic analogs of phosphate diester anions.

Thus, oligonucleotide analogs (Figure 2) were synthesized that were "isosteric" (with the same skeleton as natural oligonucleotides) and "rigid" (because they contain a ring).

However, additional building blocks are needed to control the binding affinity and polarity of these oligonucleotide analogs. This need arises because in vivo: (a) the temperature is fixed; (b) the binding of rigid isosteric analogs at the fixed temperature is tight even with short oligonucleotides (ca. 10 bases long); (c) however, such short segments usually occur more than once in the DNA of an average organism. For example, a useful "anti-sense" oligonucleotide in man would be 16 bases long and form a stable helix with a complementary natural oligonucleotide 16 bases long, but not 15 bases long, at 37°C. Rigid isosteric oligonucleotides would bind to both under these conditions.

One way to alter the binding is to incorporate different base analogs and linking skeletons into the oligonucleotide analog.

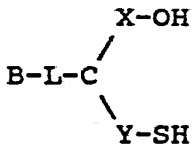
Therefore, a series of "flexible" building blocks (lacking a ring) (Figures 3 and 4) were invented. An oligonucleotide analog incorporating such flexible subunits at one position will generally bind more weakly to a complementary oligonucleotide. Likewise, base analogs other than those occurring in natural oligonucleotides are in many cases capable of forming hydrogen bonds with complementary bases, but with different overall binding affinity.

"Isosteric" flexible oligonucleotide analogs contain the same number of atoms between base analogs as between bases in natural oligonucleotides (Figure 3). Isosteric subunits in oligonucleotide analogs fit best the standard double helical structure. Incorporation of non-isosteric building blocks (Figure 4) into an oligonucleotide analog also alters the binding constant of the analog to a complementary oligonucleotide.

Further, the ability of an oligomer to pass across biological barriers (digestive system, blood vessel walls, cell membranes) is influenced by the overall polarity of the oligomer, and whether it is recognized by transport systems. Membrane permeability and transport systems are different in bacteria, lower eukaryotes, and higher eukaryotes. Thus, subunits that contribute differently to the polarity of the oligonucleotide analog, therefore influencing membrane and water solubility of the oligonucleotide analogs, are valuable.

Building blocks therefore were invented to incorporate different numbers of carbon atoms and heteroatoms in the skeleton and base analog. Examples of these are disclosed in Figures 2, 3 and 4.

Building blocks that lack a ring are described by the general formula:

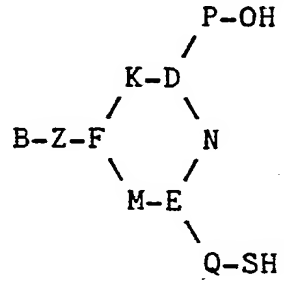


Formula 1

where B is a base analog (Figure 5) capable of forming hydrogen bonds to a base on the target oligonucleotide, L is a linking group containing $-CH_2-$ and -O- units with from 0 to 5 linking atoms (atoms in the connecting chain), C is an atom with defined chirality, X and Y are linking groups containing $-CH_2-$ and -O- units with from 0 to 6 linking atoms, with the sum of X and Y less than or equal to 8, OH is a hydroxyl group, and SH is a thiol (or mercapto) group. Preferably, the number of linking atoms in L is less than or equal to the sum of the linking atoms in X and Y.

These skeletons are classified by three numbers designating the number of atoms in the chains that link atom C with the base, the -OH group, and the SH group respectively (i.e., the number of bonds between the C and the base, the OH group, and the SH group respectively, minus 1). Heteroatoms may be present in the linking groups, and this fact is designated by including the atomic symbol as a superscript. If heteroatoms are present, they are preferably oxygen.

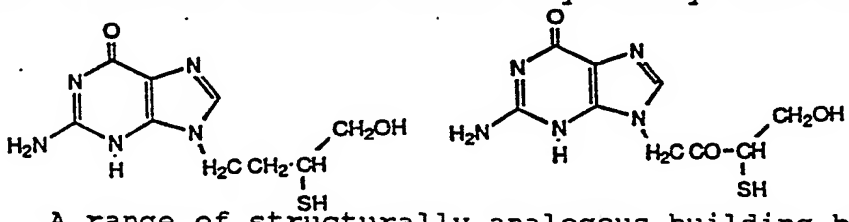
Building blocks that contain a ring are described by the general formula:



Formula 2

where B is a base analog ring system capable of forming hydrogen bonds to a natural base on a target oligonucleotide, K, M and N are linking groups containing $-CH_2-$ and -O- units with from 0 to 2 linking atoms. D, E, and F are carbon atoms with defined chirality, and P, Q and Z are linking groups containing $-CH_2-$ and -O- units with from 0 to 4 atoms.

A computer literature search found only two examples of compounds with similar formulae (Formula 3).\(12) In both, the number of linking atoms in L (2) is greater than the sum of the linking atoms in X (1) and Y (0). Thus, although these molecules might be incorporated into the oligonucleotide analogs disclosed here, oligonucleotide analogs made from these compounds should bind to natural nucleic acids only weakly if at all.



Formula 3

A range of structurally analogous building blocks is necessary to construct non-ionic oligonucleotide analogs to be applicable to different specific cases. While oligonucleotide analogs built from a single building block may have the combination of properties desired for a particular application, this will be true only fortuitously. More generally, such an oligonucleotide analog will bind to a target oligonucleotide too tightly (or too loosely) in a particular environment at a particular temperature, or will have a membrane solubility too high (or a water solubility too high) for optimal permeation of a

specific biological barrier under specific conditions.

For a specific application, the building blocks used as precursors for the oligonucleotide analog will depend on the desired application according to the following rules:

(a) The building blocks in the oligonucleotide analog are chosen based on the ability of their constituent base analogs to form hydrogen bonds with a complementary target oligonucleotide as would be predicted based on the assumption that the natural oligonucleotide and the oligonucleotide analog form double helical structures.

(b) The linker groups and base analogs in the building blocks are chosen to modulate the tightness of binding of the oligonucleotide analog to the target natural oligonucleotide, where the proportion of flexible subunits is greater for oligonucleotide analogs intended to bind to longer oligonucleotides at lower temperatures and greater ionic strength, than for oligonucleotide analogs intended to bind to shorter oligonucleotides at higher temperatures and lower ionic strength; and where the number of hydrogen bonds formed between strands is smaller for oligonucleotide analogs intended to bind to longer oligonucleotides at lower temperatures and greater ionic strength, than for oligonucleotide analogs intended to bind to shorter oligonucleotides at higher temperatures and lower ionic strength.

(c) The linker groups and base analogs in the building blocks are chosen to modulate the lipophilicity of the oligonucleotide analog, where increased number of $-CH_2-$ groups replacing -O- groups, or -CH- groups replacing -N= groups, increases

lipophilicity and decreases water solubility.

The prefered building blocks for constructing long (5-30 bases) oligonucleotide analogs for binding to complementary natural DNA and RNA are flexible. In Formula 1, the sum of linking atoms (those in the chain) of linking group L is preferably from 0 and 5, and more preferably 0, 1 or 2; of X preferably between 1 and 5, and more preferably 1, 2, or 3; and of Y preferably between 0 and 5, and more preferably 2 or 3. Most preferably, the number of linking atoms in L, X, and Y is respectively 2, 2, and 2, or 2, 3, and 1, or 2, 1, and 3, or 0, 1, and 1. Further, the sum of the linking atoms in X and Y is preferably greater than or equal to the number of linking atoms in L.

The prefered building blocks for constructing short (1-10 bases) oligonucleotide analogs that bind tightly to complementary natural DNA and RNA are rigid. Preferably, the sum of linking atoms in groups K, M, and N (referring to Formula 2) is less than 4, more preferably 2; most preferably K is either a $-CH_2$ group or an oxygen, N is $-CH_2-$, M is a bond, and C, D, and E are CH groups. Preferably, the sum of the linking atoms in P and Q is less than 6, and Z contains less than 2 linking atoms; more preferably the sum of atoms in P and Q is 3, and Z is a bond, most preferably X is selected from the group consisting of $-CH_2CH_2-$ and CH_2, and Y is selected from the group consisting of CH_2 and $-CH_2CH_2-$.

An example of an oligonucleotide analog composed from different linkers and bases is shown in Figure 6b.

Synthetic Methods

The building blocks for the synthesis of oligonucleotide

analogs are compsed of three parts, a ring system that is the precursor for the base analogs, the functional groups (generally, -SH and -OH groups), and the skeleton that links the base analogs to the functional groups. In many of the compounds disclosed here, the syntheses of different skeletons is unique to the skeleton. However, a small number of synthetic methods are satisfactory for introducing base analogs and functional groups into most skeletons. Finally, a single prefered method is disclosed here for assembling building blocks to form an oligonucleotide analog.

Coupling Reactions for Synthesis of Oligonucleotide Analogs

The oligonucleotide analogs are built by sequential couplings of building blocks. The building blocks for the synthesis of the oligonucleotide analogs consist of four parts: (a) a linking moiety bearing (b) a single sulfhydryl group (-SH) (c) a single unprotected hydroxyl group (-OH, most preferably a primary alcohol group), and (d) a suitably protected base analog. An oligonucleotide analog with the desired sequence of building blocks is synthesized by stepwise condensation of the appropriate building blocks onto a growing oligonucleotide analog chain according to the scheme in Figure 6. The sequence involves reaction of the free hydroxyl group of a growing oligomer chain with methanesulfonic acid anhydride, reaction of the resulting methanesulfonate with the thiolate anion of the next building block, and finally oxidation (if desired) of the resulting thioether to the sulfoxide or sulfone.

Depending on whether the desired product is (a) the sulfide,

(b) the sulfoxide, or (c) the sulfone, the thioether is (a) not oxidized, (b) oxidized with 1 equivalent of aqueous hydrogen peroxide at 0°C, or (c) oxidized with potassium hydrogen persulfate ($KHSO_5$).

Protecting Groups

The amine (NH_2) groups of adenine, cytosine, and guanine are preferably protected as their corresponding N-benzoyl or N-isobutyroyl amides. Amino groups on other base analogs are preferably protected as N-benzoyl amides.

Where the carbon skeleton permits the oxygen of uracil or thymidine (or a similar base analog) to form a 5 or 6 member ring via intramolecular attack of the heterocyclic ring oxygen on the activated methanesulfonate intermediate, the pyrimidine ring is preferably protected by an N-mesityl group.\(13)

Chain Initiation

Building block (10 mmol) is dissolved in anhydrous dimethylformamide (5.2 g). Subsequent reactions are run under argon. To the solution is added a methanolic solution of benzyltrimethylammonium methoxide (11.0 mmol). The mixture is stirred at room temperature (5 min), and a solution of an end group containing a reactive leaving group (in this example, methyl iodide, 11 mmol) in DMF (5 ml) is added dropwise to yield the sulfide. The mixture is then stirred for 150 min to yield the first building block (intended for the oligonucleotide analog) with its thiol group blocked as a thioether. The nature of the blocking group is chosen to increase or decrease the lipophilicity of the final oligonucleotide analog, and may be attached to a solid support or a soluble polymer. However, it must be chosen so

that it contains no unprotected functional groups that react under conditions of subsequent chain elongation.

Oxidation to Sulfoxide

The reaction mixture is cooled to 0°C, and a standardized solution of hydrogen peroxide (1.0 equivalents, 30% in water) is added. The mixture is then stirred allowed to warm slowly to room temperature over 30 minutes, and then stirred an additional 30 minutes at room temperature to yield the sulfoxide.

Oxidation to Sulfone

The reaction mixture is cooled to 0°C, and a suspension of potassium hydrogen persulfate (30 mmol, in 50 aqueous solution buffered with citrate to pH 5.0) is added. The mixture is then stirred at room temperature for 30 minutes to yield the sulfone.\(14)

Chain extension: Mesylation

The product from the previous condensation (the growing oligonucleotide chain) (1 mmol) is dissolved in dry CH_2Cl_2 (50 ml) under an atmosphere of argon. To the stirred mixture is added slowly pyrrolidinopyridine (2.4 mmol) and methanesulfonic acid anhydride (2.3 mmol). The solvents are removed by evaporation, and the residue is dissolved in ether and filtered through Kieselgel. The methanesulfonate is then purified by high performance liquid chromatography.

Chain extension: S_N2 Reaction

A specimen of the desired building block (10 mmol) is dissolved in anhydrous dimethylformamide (5.2 g). To the solution is added a methanolic solution of benzyltrimethylammonium

methoxide (11.0 mmol). The mixture is stirred at room temperature (5 min), and a solution of the methanesulfonate from the previous condensation step (11 mmol) in DMF (5 ml) is added dropwise. The reaction is stirred for 1 hour at room temperature, the solvents evaporated under vacuum (0.02 torr, 40°C), the residue redissolved in methylene chloride, and the solution extracted with water. The organic phase is dried (magnesium sulfate), the solvents removed, and the product sulfide is then purified by high performance liquid chromatography. The cycle of reactions is then repeated, with building blocks added in the desired order in each cycle. Last, protecting groups are removed, preferably in aqueous NH_4OH.

Building Blocks

The synthesis of building blocks for the oligonucleotide analogs disclosed here is divided into two parts. The first involves obtaining the skeleton of carbon and heteroatoms that will become the linking unit. This skeleton contains a chiral center. The second involves the introduction of nucleoside base analogs and functional groups onto this skeleton to form the building blocks for the synthesis of the oligonucleotide analogs.

Introduction of the Thiol Group

Except in cases where the skeleton is derived from cystine, homocystine, or other molecules that already bear a thiol group and are readily obtained, the prefered method for introducing a thiol group is the Mitsunobu reaction, involving the conversion of a hydroxyl group to a CH_3CO-S- group through the reaction of triphenylphosphine, diethylazodicarboxylate, and thioacetic acid. As the acetate derivative, the thiol group is protected against

oxidation, yet can be deprotected by mild base or by reduction (e.g., with lithium triethylborohydride). For skeletons that can be obtained commercially already bearing a thiol group, this group is preferably protected as a dimeric disulfide, and the thiol is preferably generated by reduction prior to coupling.

Introduction of the Base Analog Ring System

Skeletons are obtained so that the carbon atom intended to bear the base analog is functionalized either with (a) an amino group, (b) a hydroxyl group, (c) a halogen group, (d) a $O-CH_2-L$ moiety, where L is a leaving group, (e) an epoxide, or (f) a ring system. Depending on the functional group involved and the nature of the ring system desired, one of six different methods is used to append the ring system:

(a) Where the skeleton already contains an amino group at the appropriate position, the ring system is built up by a series of condensation reactions, different for each base. The prefered method for constructing thymidine and uracil rings is by reaction with 2-methyl-3-ethoxyacryoyl isocyanate and 3-ethoxyacryoyl isocyanate respectively, followed by cyclization in dilute acid.\(15) Adenine, guanine, and other purine rings can also be built up around an amino groups pre-existing in the skeleton.

(b) Where the skeleton contains a hydroxyl group at the appropriate position, the ring system is introduced by the Mitsunobu reaction of a corresponding base or base analog described below. This is the prefered method for introducing 6-chloropurine rings as precursors to adenine ring systems.

(c) Where the skeleton contains a halogen group at the

appropriate position, the ring system is introduced by a nucleophilic substitution reaction with an appropriately protected heterocyclic ring or ring precursor. This is the prefered method for adding the 2-amino-6-chloropurine base analog to such skeletons.

(d) Where the skeleton bears a $O-CH_2-L$ moiety, where L is a leaving group, the ring system is introduced by reaction of an appropriately protected heterocyclic ring or ring precursor under electrophilic conditions. These protected heterocyclic rings or their precursors are already well known in the prior art. Thus, the prefered method for introducing a uracil, thymidine, adenine, guanine, and tubercidin, is by reaction of bistrimethylsilyloxypyrimidine, 2-trimethylsilyloxy-4-trimethylsilylaminopyrimidine, N-benzoyladenine, N-benzoylguanine, or N-benzoylaminopyrrolopyrimidine with a O-CHR-Cl moiety on a skeleton.

(e) Where the skeleton bears an epoxide group, the prefered method for introducing a base is by nucleophilic substitution by a ring system at the less hindered site of the epoxide.\(16)

(f) Where the skeleton already bears a ring system, standard methods familiar to those skilled in the art enable the conversion of one ring system to another. Thus, one method for constructing cytidine rings is by conversion of uracil rings. The prefered method for constructing an adenine ring system from a 6-chloropurine ring system is reaction with methanolic ammonia. The prefered method for constructing a guanine ring system from a 2-amino-4-chloropurine ring system is hydrolysis in dilute aqueous

acid.

Construction of the Carbon Skeleton

Precursors for the flexible skeletons shown in Figures 3 and 4 are shown in Figure 4. Methods for constructing building blocks from a representative sample of these precursors are disclosed in the Examples using the general synthetic routes for introducing functional groups outlined above. These Examples teach one skilled in the art to construct appropriately functionalized flexible building blocks from the precursors shown in the Figures. Detailed procedures for constructing rigid skeletons (which involve more complicated synthetic transformations) are given in Examples 1 and 2.

Examples

Abbreviations:
THF: tetrahydrofuran
EtOAc: ethyl acetate
RT: room temperature
NMR: nuclear magnetic resonance spectroscopy
GC: gas chromatography
IR: infrared spectrum
DEAD: diethylazodicarboxylate
THP: tetrahydropyranyl
Bz: benzoyl (Phenyl-CO-)
BDS: tert-butyldimethylsilyl
Bn: benzyl (Phenyl-CH_2)
Ac: acetyl (CH_3CO-)
Pv: pivaloyl ($(CH_3)_3CCO$-

Examples

Example 1 (Figure 7)

Rigid, Isosteric Building Block, Heteroatom in Linker

Ethyl 3-Acetoxyacrylate (1)

A mixture of EtOAc (100.0 g, 1.136 mol) and ethyl formate (80.7 g, 1.136 mol) was slowly added with stirring at RT to sodium wire (26.0 g, 1.130 mol) in dry ether (400 ml). The reaction mixture was stirred for 20 hours, and the sodium salt of ethyl-3-hydroxyacrylate was isolated by centrifugation as brownish precipitate (yield after drying, 92 g). The sodium salt was finely ground, suspended in ether (400 ml), and cooled to 0°C. Acetyl chloride (55.25 g, 0.70 4 mol) was added dropwise at 0°C to this suspension. The reaction mixture was stirred at RT for 24 hours, then poured into aqueous saturated sodium bicarbonate (200 ml) at 0°C. The organic phase was separated, washed with water (5 x 200 ml), dried (magnesium sulfate) and the solvents evaporated. The resulting oil was distilled under vacuum to yield ethyl-3-acetoxyacrylate (20% overall yield) in a cis:trans mixture

(1:1.4 by NMR and gas chromatography).

1-Acetoxy-2-carboethoxycyclohex-4-ene (cis and trans) 3 and 2

Ethyl 3-acetoxyacrylate (1, 11.0 g, 69.6 mmol) and butadiene (30.0 ml, 0.8 mol) were dissolved in toluene (distilled from calcium hydride, 25 ml); the mixture was placed in a 500 ml autoclave at -78°C. The autoclave was sealed, and then heated for 15 hours at 170°C. The autoclave was cooled and opened, the contents centrifuged, and the gummy residue extracted with toluene (3 x 100 ml). The toluene was removed under vacuum, and the mixture of cis and trans 1-acetoxy-2-carboethoxycyclohex-4-ene (3 and 2) was isolated by distillation (101°C at 2 torr).

Trans 1-Hydroxy-2-carboethoxycyclohex-4-ene 2a

Sodium hydride (200 mg) was added to a mixture of the cis and trans isomers of ethyl 1-acetoxy-2-carboethoxycyclohex-4-ene (1.60 g, 7.55 mmol) in absolute ethanol (50 ml), and the mixture was stirred for 1 hour at RT. The reaction mixture was neutralized with Dowex W 50 cation exchange resin (acid form), the resin removed by filtration, the solvents removed under vacuum, and the residue chromatographed on silica gel (hexane:EtOAc 7:3, Rf=0.40) to yield 1.2 g (94%) trans 1-hydroxy-2-carboethoxycyclohex-4-ene (IR CCl_4, 1732 cm^{-1}).

Ethyl cis-2-benzoyloxy-cyclohex-4-enecarboxylate 4

DEAD (2.06 g, 11.8 mmol) was added dropwise to a solution of triphenylphosphine (3.08 g, 11.8 mmol) in THF (35 ml) at 0°C over a period of 5 min. Trans 1-Hydroxy-2-

carboethoxycyclohex-4-ene 2 (1.00 g, 5.88 mmol in 5 ml THF) was then added slowly, the mixture was stirred for 5 min, and then benzoic acid (1.44 g, 11.8 mmol, in 5 ml THF) was added. The mixture was stirred at RT for 2 hours. Solvents were evapoated, an the residue purified by chromatography on silica gel (EtOAc:hexane 2:8 as eluant). The product was then distilled under vacuum to yield ethyl cis-2-benzoyloxy-cyclohex-4-enecarboxylate (4, 1.1 g, 71%).

(1S,2R)-1-Benzoyloxycyclohex-4-ene-2-carboxylic acid

Pig liver esterase (16 mg, 2000 units) was added to ethyl cis-2-benzoyloxy-cyclohex-4-enecarboxylate (4) suspended in a mixture of t-butanol and water (1:9) at pH 7, and the progress of the enzymic hydrolysis was followed with an autotitrator (maintaining the pH at 7) with 0.5 N NaOH). After 36% of the starting material had been hydrolyzed, the reaction was quenched with CH_2Cl_2 (3 ml) and extracted with ether (3 x 30 ml). The ethereal layers were dried (magnesium sulfate), and the solvent removed under vacuum to yield 1.9 g (63%) of a mixture of (1S,2R)- and (1R,2S) 1-benzoyloxy-2-carboethoxycyclohex-4-ene. The pH of the aqueous phase was adjusted to 2 and extracted with ether (3 x 30 ml). The ethereal layers were dried (magnesium sulfate), and the solvent removed under vacuum to yield 982 mg (35.8%) crude (1S,2R)-1-benzoyloxycyclohex-4-ene-2-carboxylic acid (5). This was shown to be essentially free of its enantiomer via gas chromatographic analysis of the 1-norborneol ester, and by NMR in the presence of a europium chiral shift reagent.

(1R,2S)-1-Benzoyloxycyclohex-4-ene-2-carboxylic acid 5

Pig liver esterase (6 mg, 800 units) was added to cis 1-benzoyloxy-2-carboethoxycyclohex-4-ene suspended in a mixture of t-butanol and water (1:9) at pH 7, and the mixture incubated at RT for 15 hours. Following work-up as described above, the first ethereal extract yielded 1.46 g (48%) of (1R,2S) 1-benzoyloxy-2-carboethoxycyclohex-4-ene >97% enantiomerically pure ($[\alpha]_D$, (c 3.1, acetone) = $+105.6^{o}$.

Methyl cis-2-benzoyloxy-cyclohex-4-enecarboxylate 6

A solution of 1-benzoyloxycyclohex-4-ene-2-carboxylic acid (5, 160 mg, 0.645 mmol) in ether (5 ml) is treated with a solution of diazomethane (excess) in ether. The solvent was removed under vacuum to yield methyl cis-2-benzoyloxy-cyclohex-4-enecarboxylate (6, 168 mg, 100%) as a colorless oil. When enantiomerically pure starting material was used, the product of this reaction was >97% enantiomerically pure. $[\alpha]_D$ (c 6.35, acetone) = -89.5^{o} for the 1S,2R isomer).

cis-2-Hydroxymethyl-cyclohex-4-eneol 7

Methyl cis-2-benzoyloxy-cyclohex-4-enecarboxylate (6, 1.1 g, 4.19 mmol) was dissolved in THF (10 ml), and the solution added to a dispersion of lithium aluminum hydride (0.35 g, 9.15 mmol) in THF (30 ml) at $-78^{o}C$. The mixture was warmed to RT and stirred for 5 hours, cooled to $-15^{o}C$, and diluted successively with water (0.35 ml), 15% NaOH (0.35 ml), and water (1.05 ml). The precipitate was removed by filtration, washed with EtOAc, and the combined filtrates evaporated under vacuum. The residual oil was purified by chromatography on

silica gel (EtOAc, Rf=0.28) to yield cis 2-hydroxymethyl-cyclohex-4-eneol (7, 419 mg, 78%) as a colorless oil.

2-Pivaloyloxymethyl-cyclohex-4-eneol 8

Pivaloyl chloride (1.036 g) was added at -20°C to a solution of 2-hydroxymethyl-cyclohex-4-eneol (7, 1.00 g, 7.81 mmol) in pyridine (10 ml). The reaction mixture was kept at -20°C for 20 hours, diluted with methanol and ether (50 ml), and the organic layer extracted with 20 ml portions of 10% HCl containing copper sulfate. The ethereal layer was dried (magnesium sulfate), the solvent removed under vacuum, and the residue chromatographed on silica gel (hexane:EtOAc 7:3, Rf = 0.43) to yield 2-pivaloyloxymethyl-cyclohex-4-eneol (8, 1.54 g, 93%) as a colorless oil. (IR, CCl_4 1730 cm^{-1}).

3,4-trans 1-Methoxy-3-pivaloyloxymethyl-4-(2'-hydroxyethyl)-tetrahydrofuran 9 and 1-Methoxy-3-pivaloyloxymethyl-4-(2',2'-dimethoxyethyl)-tetrahydrofuran.

2-Pivaloyloxymethyl-cyclohex-4-eneol (8, 6.327 g, 29.8 mmol) was dissolved in methanol (250 ml), cooled to -78°C, and then treated with ozone (ca. 30 min) until the solution was blue. After excess ozone was removed in a stream of nitrogen (45 min), dimethylsulfide (10 ml) was added, and the reaction was warmed to RT and stirred in the dark for 7 days. Analysis by gas chromatography showed a 3:1 mixture of the 2' aldehyde and its corresponding dimethyl acetal. The mixture was cooled to 0°C, and $NaBH_4$ (1.00 g) was added in small portions. After 20 min., 5% HCl (20 ml) was added, half of the methanol

evaporated, the mixture was diluted with water (100 ml), and the mixture was extracted with CH_2Cl_2 (3 x 100 ml). The organic layer was dried (magnesium sulfate), the solvent evaporated under vacuum, and the residue chromatographed on silica gel (hexane:EtOAc 6:4) to yield a mixture of 3,4-trans 1-methoxy-3-pivaloyloxymethyl-4-(2'-hydroxyethyl)-tetrahydrofuran (9, 4.85 g, 62%, IR, CCl_4, 1732 cm^{-1}) and 1-methoxy-3-pivaloyloxymethyl-4-(2',2'-dimethoxyethyl)-tetrahydrofuran (1.845 g, 20%, IR, CCl_4, 1732 cm^{-1}) as colorless oils and as mixtures of anomers.

exo-6-Pivaloyloxymethyl-2,8-dioxa-[1.2.3]-bicyclooctane 10

A solution of 3,4-trans 1-methoxy-3-pivaloyloxymethyl-4-(2'-hydroxyethyl)-tetrahydrofuran (9, 500 mg) in toluene (10 ml) with Dowex W 50 cation exchange resin (acid form) was refluxed for 3 hours. The resin was removed by filtration, and the solvent evaporated under vacuum to yield exo-6-pivaloyloxymethyl-2,8-dioxa-[1.2.3]-bicyclooctane (10, 434 mg, 99%) as colorless crystals (from hexane). NMR ($CDCl_3$) 1.20 (s, 9H), 1.57 (s, br, 1H), 1.67 (ddd, J=14,3,2.5 Hz, 1H), 2.30 (m, 2H), 2.51 (m, 1H), 3.81-4.08 (m, 4H), 4.32 (s, br, 1H), 5.44 (d, J=5 Hz)

3',5'-Bishomo-2-deoxyuridine-3'O-pivalate 11a

To a solution of bis-trimethylsilyloxypyrimidine in acetonitrile was added exo-6-pivaloyloxymethyl-2,8-dioxa-[1.2.3]-bicyclooctane (10, 200 mg, 0.877 mmol) in acetonitrile (1 ml). A solution of trimethylsilyltriflate (0.191 ml, 1.2 equivalents) in dichloroethane (1 ml) was then added, and the

mixture stirred at RT for 15 hours. The mixture was then poured onto ice cold saturated aqueous sodium bicarbonate, the aqueous layer was extracted with CH_2Cl_2 (3 x 10 ml), the solvent removed under vacuum, and the residue chromatographed on silica gel ($CHCl_3$:methanol 9:1, Rf 0.20) to yield a mixture of anomers of 3',5'bishomo-2-deoxyuridine-3'O-pivalate as a white foam (11a, 1:1 mixture, 156 mg, 52%). The beta anomer (11a) is the preferred anomer. NMR ($CDCl_3$) 1.20 (2 s, 9H), 1.78-2.50, 2.74 (mm, 6H), 3.84, 4.00, 4.13 (3 m, 5H), 5.77 (d, J=10 Hz, 1H), 6.17 (m, 1H), 7.46 (2 d, J=10 Hz, 1H), 9.59 (s, br, 1H, exchangeable). Anal calc. for $C_{16}H_{24}N_2O_6$: C, 56.46%; H, 7.11%; N, 8.23%. Found C, 55.97%; H, 6.98%; N, 8.00%.

5'-Deoxy-5'-methylcarboxythio-3',5'-bishomo-2-deoxyuridine-3'O-pivalate 12a

DEAD (0.03 ml, 0.18 mmol) was added at 0°C to a solution of triphenylphosphine (47 mg, 0.18 mmol) in THF (0.5 ml). The mixture is stirred for 30 min. 3',5'-bishomo-2-deoxyuridine-3'O-pivalate (11a, 0.09 mmol) dissolved in THF (0.5 ml) is then added together with thioacetic acid (0.013 ml, 0.18 mmol)) in THF (0.5 ml). The reaction mixture is stirred at 0°C for 1 hour, and then for another hour at RT. The solvents are then evaporated under vacuum, and the residue chromatographed on silica gel (20 g, EtOAc eluant) to yield 5'-deoxy-5'-methylcarboxythio-3',5'-bishomouridine-3'O-pivalate.

This compound could be conveniently stored. Free thiol-alcohol building block (13a) for use in the synthesis of

oligonucleotide analogs is prepared immediately prior to coupling by reduction of 12a with lithium triethylborohydride in THF.

Example 2 (Figure 7)

Rigid, Isosteric Building Block, Heteroatom in Linker

3',5'-Bishomo-2-deoxy-N-benzoyladenine-3'O-pivalate 11b

N-Benzoyladenine (483 mg, 2 mmol) was suspended in acetonitrile (5 ml), and N-methyl-N-trimethylsilyltrifluoroacetamide (1.31 ml, 6.34 mmol) was added to give a clear solution. Exo-6-pivaloyloxymethyl-2,8-dioxa-[1.2.3]-bicyclooctane (10, 500 mg, 1.92 mmol) (from Example 1) was then added, and the mixture stirred for 1 hour at RT. Stannic chloride (0.32 ml, 1.5 equivalents) was then added, and stirring continued for 5 hours. The reaction was quenched with cold saturated sodium bicarbonate (10 ml), and the products extracted with EtOAc and flashed with methanol chloroform (1:9) as eluant to yield N-benzoyl 3',5'-bishomo-2-deoxyadenosine-3'O-pivalate 11b.

5'-Deoxy-5'-methylcarboxythio-3',5'-Bishomo-2-deoxy-N-benzoyladenosine-3'O-pivalate 12b

DEAD (0.03 ml, 0.18 mmol) was added at 0°C to a solution of triphenylphosphine (47 mg, 0.18 mmol) in THF (0.5 ml). The mixture is stirred for 30 min. 3',5'-Bishomo-2-deoxy-N-benzoyladenosine-3'O-pivalate (11b, 0.09 mmol) dissolved in THF (0.5 ml) is then added together with thioacetic acid (0.013 ml, 0.18 mmol)) in THF (0.5 ml). The reaction mixture

is stirred at 0°C for 1 hour, and then for another hour at RT. The solvents are then evaporated under vacuum, and the residue chromatographed on silica gel (20 g, EtOAc eluant) to yield 5'-deoxy-5'-methylcarboxythio-3',5'-bishomo-2-deoxy-N-benzoyladenosine-3'O-pivalate 12b.

This compound could be conveniently stored. Free thiol-alcohol building block (13b) is prepared immediately prior to coupling by reduction of 12b with lithium triethylborohydride in THF.

Example 3 (Figure 8)

Rigid, Isosteric Building Block, No Heteroatom in Linker

exo Norbornene-2-carboxaldehyde 15

Lithium hydroxide (960 mg, 20 mmol, in 20 ml water) was added to a solution of norbornene-2-carboxaldehyde (14, Fluka, 24.4 g, 0.2 mol in 500 ml of THF), and the mixture was stirred overnight under reflux. The reaction mixture was then neutralized (to pH 7.5) at 0°C with concentrated HCl, and the THF/water azeotrope removed under reduced pressure. The residue was chromatographed on Kieselgel (pentane:ether (30:1) as eluant) to yield exo norbornene-2-carboxaldehyde (15, 11.0 g, 45%).

exo Norbornene-2-methanol 16

A solution of sodium borohydride (1.7 g, 45 mmol) in 20 ml of 2 N NaOH was added dropwise at 0°C over a period of 1 hour to a solution of exo norbornene-2-carboxaldehyde (15, 11 g, 90 mmol) in methanol (50 ml). The mixture was allowed to

warm to RT, and was stirred for 1 hour. The progress of the reaction was monitored by thin layer chromatography. After the reaction was complete, the mixture was acidified at 0°C by slow addition of concentrated HCl, and the product extracted twice with ether (500 ml). The ether was washed with aqueous sodium bicarbonate, dried (Mg_2SO_4), and evaporated to yield exo norbornene-2-methanol (16, 10.8 g, 97% yield).

exo Norbornene-2-methanol acetate 17

Exo norbornene-2-methanol (16, 9.82 g, 79 mmol) was dissolved in pyridine (13 ml) containing dimethylaminopyridine (0.75 g, 1.6 mmol). The mixture was cooled to 0°C under argon, and acetic anhydride (10 ml, 103 mmol) was added dropwise. After addition, the solution was allowed to stir one half hour at RT. Ice (50 g) was added, and the mixture was acidified at 0°C, and extracted twice with ether. The extracts were washed (dilute HCl, then sodium bicarbonate), dried and evaporated. Exo norbornene-2-methanol acetate (17, 10.3 g, 56 mmol, 71%) was recovered by distillation under vacuum (87°C at 12 torr).

exo 5- and 6-Hydroxy-2-exo-norbornanmethanol acetate 18 and 19

Diborane, generated by the treatment of sodium borohydride (2.3 g, 67 mmol) in THF (40 ml) with trifluoroboron etherate (15 ml) over a period of 30 min was passed into a solution of exo-norbornene-2-methanol acetate (17, 10.3 g, 62 mmol) in THF (60 ml) at 0°C. After the addition was complete, the reaction was quenched with water

(10 ml) and 2 N NaOH (10 ml). Then hydrogen peroxide (8 ml, 30%) was added at 0° C. After one hour at 50°C, sodium chloride was added and the mixture extracted with EtOAc. Normal work up yielded a crude mixture of exo 5- and 6-hydroxy-exo-norbornene-2-methanol acetates (18 and 19, 12 g), which were not separated at this point.

This same intermediate can be prepared in optically active form from optically active exo t-butyl 5-norbornene-2-carboxylate synthesized via an asymmetric Diels-Alder reaction (Oppolzer, W.; Chapuis, C.; Kelly, M. J., Helv. Chem. Acta 1983, 66, 2358-2361) followed by reduction with $LiEt_3BH$ and hydroboration by the method described above.

5- and 6-keto-exo-norbornane-2-methanol acetate 20 and 21

Pyridinium chlorochromate (21.5 g, 100 mmol) was stirred with Celite (15 g) and methylene chloride (140 ml) at RT. To the suspension was added a mixture of 5 and 6 hydroxy-exo-norbornane-2-methanol acetate (18 and 19, 12 g, 62 mmol) in methylene chloride (70 mmol) dropwise over a period of 30 min. The mixture was stirred for an hour at RT, at which point the dark brown suspension was filtered through silica gel (60 g). Chromatography yielded a crude mixture of 5- and 6-keto--exo-norbornane-2-methanol acetate (20 and 21, 12.5 g). This mixture was separated by silica gel chromatography (column diameter 5 cm x 45 cm, ca. 400 g Kieselgel) using pentane:ether (1:1) as an elution solvent. After distillation, the desired 5-keto-exo-norbornane-2-methanol acetate crystallized (20, 4.36 g, 40% yield) upon cooling.

3-Keto-2-oxa-6-exo-bicyclo-[3.2.1]-octanmethanol acetate 22

5-Keto-exo-norbornane-2-methanol acetate (21, 3.3 g, 18 mmol) was stirred with sodium acetate (9 g, water-free), hydrogen peroxide (27 ml, 30%), and acetic acid (9 ml) at 5°C in the dark for 40 hours. The reaction was quenched by the addition of sodium sulfite (50 ml of a 10% aqueous solution) at 0°C. The product was extracted twice with EtOAc (600 ml). The extract was washed with the sodium sulfite solution, then with sodium bicarbonate solution (50 ml, saturated), then with sodium chloride solution (saturated), dried and evaporated. The product was chromatographed on silica (380 g, ether) to yield 3-keto-2-oxa-6-exo-bicyclo-[3.2.1.]-octanmethanol acetate (22, 2.4 g, 12 mmol, 67%, mp 55-56°C).

Methyl 2-trans-hydroxymethyl-4-cis-hydroxycyclopentaneacetate 23

3-Keto-2-oxa-6-exo-bicyclo-[3.2.1.]-octanmethanol acetate (22, 2.4 g, 12 mmol) was dissolved in methanol (100 ml) at 0°C, and a solution of sodium metal (560 mg, 24 mmol) in methanol (50 ml) was added. After refluxing overnight, the mixture was acidified at 0°C with acetic acid (to pH 8.5). After filtration and evaporation, the product was chromatographed on silica (350 g) with ether:ethanol (19:1) as eluant to yield methyl-2-hydroxymethyl-4-hydroxycyclopentane-1-acetate (23, 2.12 g, 94% yield) as a colorless oil.

Methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-hydroxycyclopentaneacetate 24

Methyl 2-trans-hydroxymethyl-4-cis-hydroxycyclopentaneacetate (23, 430 mg, 2.3 mmol) and imidazole (340 mg, 5mmol) was dissolved in dimethylformamide (2.5 ml) at 0°C. To the mixture was added t-butyldiphenylsilyl chloride (692 mg, 0.64 ml, 2.52 mmol) at 0°C. The mixture was then warmed to RT and stirred for 2 hours. The excess silyl chloride was hydrolyzed with water, and the product extracted twice with EtOAc. The extracts were washed with saturated sodium chloride solution, dried, and evaporated to yield methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-hydroxycyclopentaneacetate (24, 640 mg, 67%) as a colorless liquid.

Methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-trans-benzoyloxycyclopentaneacetate 25

Triphenylphosphine (2.9 g, 11.1 mmol) and benzoic acid (1.35 g, 11.1 mmol) in THF (74 ml) were added to a solution of methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-hydroxycyclopentaneacetate (24, 3.16 g, 7.4 mmol). The mixture was cooled to 0°C with stirring, and then DEAD (1.87 ml, 11.1 mol, in 15 ml THF) was added. The mixture was stirred a half hour, yielding a clear slightly yellow solution. The solvents were evaporated under vacuum, and the residue chromatographed on silica gel (pentane:ether (2:1) as eluant) to yield methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-trans-benzoyloxycyclopentaneacetate (25, 4.01 g).

Methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-trans-hydroxycyclopentaneacetate 26

To a solution of methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-trans-benzoyloxycyclopentaneacetate (25, 4.01 g, 7.4 mmol) in methanol (35 ml) at 0°C was added under argon a solution of sodium metal (0.19 g, 7.7 mmol) in methanol (25 ml). The mixture was stirred under argon overnight. The pH of the mixture was then adjusted to 8.5 with acetic acid, the mixture filtered, the solvents evaporated, and the products chromatographed on silica with pentane:ether (1:2) as eluant to yield methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-trans-hydroxycyclopentaneacetate (26, 2.74 g, 87% yield).

Methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(6-chloropurin-9-yl)-cyclopentaneacetate 27

To a solution of triphenylphosphine (730 mg, 2.8 mmol) and 6-chloropurine (430 mg, 2.8 mmol) in dry THF (16 ml) was added a solution of DEAD (0.42 ml, 2.8 mmol) in dry THF (3.5 ml). The mixture was stirred for 2 hours at RT. To the solution was added methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-trans-hydroxycyclopentaneacetate (26, 800 mg, 1.9 mmol) in THF (9 ml). After the reaction was complete, the solvents were evaporated, and the residue purified by chromatography on silica (60 g) with pentane:ether (1:5) as eluant to yield methyl 2-trans-t-butyldiphenylsilyloxymethyl)-4-cis-(6-chloropurin-9-yl) cyclopentaneacetate (27, 820 mg, 78%).

Methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(aden-9-

yl)-cyclopentaneacetate 28

Aqueous ammonium hydroxide (25%, 40 ml) was added to a solution of methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(6-chloropurin-9-yl)-cyclopentaneacetate (27, 480 mg, 0.85 mmol) in dioxane (40 ml) at RT. Air was excluded with a balloon filled with NH_3. After 30 min, the mixture was heated at 60°C, and stirred for 24 hours. Dioxane (100 ml) was then added, and the azeotrope removed by evaporation. The residue was chromatographed on Kieselgel using ether:ethanol (4:1) as eluant, to yield methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(aden-9-yl)-cyclopentaneacetate (28, 330 mg, 71%).

Methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(N.N-dibenzoyladen-9-yl)-cyclopentaneacetate 29

Benzoyl chloride (0.6 ml, 4.7 mmol) was added slowly to a solution of methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(aden-9-yl)-cyclopentaneacetate (28, 318 mg, 0.59 mmol) in pyridine (4 ml) and CH_2Cl_2 at 0°C. The mixture was stirred 2 hours at RT. The excess benzoyl chloride was hydrolyzed with water at 0°C, and the product was extracted twice with ether. The extracts were washed with an aqueous solution of copper sulfate (10%) and brine, dried, and evaporated. After silica chromatography with ether as eluant, methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(N,N-dibenzoyladen-9-yl)-cyclopentaneacetate was isolated (29, 381 mg, 86%) as a colorless foam.

2-(Trans-t-butyldiphenylsilyloxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanol 30

A solution of lithium triethylborohydride (1 M in THF, 0.85 ml, 0.85 mmol) was added over a period of a half hour to a solution of methyl 2-trans-t-butyldiphenylsilyloxymethyl-4-cis-(N.N-dibenzoyladen-9-yl)-cyclopentaneacetate (129 mg, 0.17 mmol) in dry THF (3 ml) at -18°C under argon. The mixture was stirred for 30 min at -10°C. More reducing agent (0.2 ml) was then added at 0°C, and stirring continued at RT for another half hour. The reaction mixture was then hydrolyzed with saturated ammonium chloride (0.5 ml), evaporated, and the residue chromatographed on silica gel (ether:ethanol 9:1 as eluant) to yield 2-(trans-t-butyldiphenylsilyloxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanol (30, 83 mg, 78%).

2-(Trans-t-butyldiphenylsilyloxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanethiol S-acetate 31

DEAD (0.03 ml, 0.18 mmol) was added to a solution of triphenylphosphine (47 mg, 0.18 mmol) in THF (0.5 ml) at 0°C, and the mixture was stirred for 30 min. Solutions of 2-(trans-t-butyldiphenylsilyloxymethyl)-4-(cis-N-benzoyladen-9-yl) cyclopentaneethanol (30, 55 mg, 0.09 mmol) in THF (0.5 ml) and thioacetic acid (0.013 ml, 0.18 mmol) in THF (0.5 ml) were then added. The reaction mixture was stirred at 0°C for 1 hour, and then for another hour at RT. The solvents were then evaporated, and the residue chromatographed on silica gel (20 g) with EtOAc as eluant, to yield 2-(trans-t-butyldiphenylsilyloxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanethiol S-acetate (31, 48 mg, 80%) as a colorless foam.

2-(Trans-t-hydroxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanethiol S-acetate 32

Tetrabutylammonium fluoride (trihydrate, 70 mg, 0.22 mmol) was added to 2-(trans-t-butyldiphenylsilyloxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanethiol S-acetate (31, 98 mg, 0.145 mmol) dissolved in dry THF (3 ml). The mixture was stirred at RT for 15 hours. The solvents were then evaporated under vacuum, and the residue chromatographed on silica gel (ether:ethanol 4:1 as eluant) to yield 2-(trans-t-hydroxymethyl)-4-cis-(N-benzoyladen-9-yl)-cyclopentaneethanethiol S-acetate (32, 90 mg, 77%) as a white foam. NMR (DMSO-d_6) 1.57-1.70 (m, 1H); 1.79-2.31 (m, 6H); 2.33 (s, 3H); 2.39-2.46 (m, 1H); 2.84-2.91 (m, 2H); 3.38-3.49 (m, 2H); 4.68-4.72(m, 1H); 4.87-4.98 (m, 1H); 7.52-7.57 (m, 2H); 7.62-7.67(m, 1H); 8.04-8.06 (m, 2H); 8.60 (s, 1H); 8.73 (s, 1H); 11.16 (s, br., 1H). Anal calc. for $C_{22}H_{25}N_5O_3S$ C 60.12%, H 5.73%, N 15.93%; found C 59.12%, H 5.74%, N 15.72%.

This compound could be conveniently stored. Free thiol-alcohol building block (33) is prepared immediately prior to coupling by hydrolysis of this compound under basic conditions.

Example 4 (Figure 9)

Rigid, Non-isosteric, Heteroatom in Linker

N-Benzoyl-3-O-methylcarboxyethyl-2'-deoxycytosine

A standardized solution of the dimethylsulfoxide anion in dimethylsulfoxide (5.1 mmol) is added to a solution of N-

benzoyl-5'-dimethoxytrityl-2-deoxycytosine (34, Aldrich, 5 mmol) in anhydrous dimethylsulfoxide (10 ml) at 0°C. Methyl bromoacetate (5.2 mmol) is then slowly added to this reaction mixture over a period of 30 min. The reaction mixture is then allowed to warm to room temperature overnight, is quenched with dilute aqueous acid, and N-benzoyl-3-O-methylcarboxyethyl-2'-deoxycytosine (35) is isolated by extraction into ether.

N-benzoyl-3-O-methylcarboxyethyl-2'5'-dideoxy-5'mercaptocytosine S-acetate 36

DEAD (0.03 ml, 0.18 mmol) is added at 0°C to a solution of triphenylphosphine (47 mg, 0.18 mmol) in THF (0.5 ml). The mixture is stirred for 30 min. The product from the previous reaction step (35, 0.09 mmol) in THF (0.5 ml) and thioacetic acid (0.013 ml, 0.18 mmol)) in THF (0.5 ml) are then added to the solution. The reaction mixture is stirred at 0°C for 1 hour, and then for another hour at RT. The solvents are then evaporated under vacuum, and the residue chromatographed on silica gel (20 g, EtOAc eluant) to yield the S-acetate of N-benzoyl-3-O-methylcarboxyethyl-2',5'-dideoxy-5-mercaptocytosine (36).

This compound can be conveniently stored, and converted to the free thiol-alcohol building block (37) immediately before coupling either by reduction with lithium borohydride in THF or by hydrolysis in base.

Example 5 (Figure 10)

Non-rigid, Isosteric, Heteroatom in Linker

5-Benzyloxy-1,3-pentanediol 39

To a solution of methyl 3-(S)-hydroxy-5-benzyloxypentanoate (38, 183 mmol)\(17) in anhydrous THF (1 l) is added lithium borohydride (7.7 g, excess) at 0°C over 15 min. The reaction mixture is stirred at RT for 30 minutes, and then refluxed for 6 hours. The mixture is then cooled to 0°C, diluted with water (60 ml), and stirred overnight at RT. A white precipitate is removed by filtration, the solvent removed in vacuum, and the residue distilled under vacuum to yield 5-benzyloxy-1,3-pentanediol 39.

1-Pivaloyloxy-5-benzyloxy-3-pentanol 40

Pivaloyl chloride (2.070 ml, 17.1 mmol) was added slowly to a solution of 5-benzyloxy-1,3-pentanediol (39, 16.3 mmol) in anhydrous pyridine (35 ml) at -18°C (dry ice-acetone bath). The mixture was kept without stirring in a -20° freezer for 15 hours. The reaction mixture was then diluted with methanol (1 ml) and CH_2Cl_2 (50 ml), and extracted with 10% HCl containing copper sulfate (10 g/l) until the blue color in the organic layer was removed. The aqueous layer was re-extracted with methylene chloride, the combined organic layers are dried (magnesium sulfate), the solvent evaporated, and 1-pivaloyloxy-5-benzyloxy-3-pentanol (40) isolated as a colorless oil by flash chromatography on silica gel (hexane/EtOAc 7:3 as eluant; yield 4.05 g, 86% as a colorless oil).

1-Pivaloyloxy-5-benzyloxy-3-O-chloromethyloxypentane 41

1-Pivaloyloxy-5-benzyloxy-3-pentanol (40, 294 mg, 1.0 mmol) was dissolved in dichloroethane (3 ml) together with paraformaldehyde (600 mg, 2.0 mmol). The solution was cooled to 0°C, and HCl gas was passed through the solution until all of the solid had dissolved (ca. 3.5 hours). The clear solution was dried (magnesium sulfate) and the solvent removed under vacuum, to yield 1-pivaloyloxy-5-benzyloxy-3-O-chloromethyloxypentane (41)
in essentially quantitative yield as a clear oil.

1'-Pivaloyloxy-5'-benzyloxy-3'-((6-chloro)purin-9-yl)-methyloxypentane 42a

6-Chloropurine (663 mg, 3.7 mmol) was dissolved in a mixture of dimethylformamide (10 ml) and triethylamine (0.57 ml). The mixture was cooled to 0°C, and as solution of 1-pivaloyloxy-5-benzyloxy-3-O-chloromethyloxypentane (41, from 1.09 g of 1-pivaloyloxy-5-benzyloxy-3-pentanol) in dry dimethylformamide (10 ml) was added slowly over a period of 15 min. After addition was complete, the mixture was allowed to warm to RT overnight. The solvent was removed under vacuum, and the residue purified by flash chromatography on silica gel to yield 880 mg (52%) of a colorless oil consisting of a 20:1 mixture of the N^9 (42a) and (presumably) the N^7 isomer of the desired product.

1'-Pivaloyloxy-5'-benzyloxy-3'-(aden-9-yl)-methyloxypentane 43a

A solution of 1'-pivaloyloxy-5'-benzyloxy-3'-((6-

chloro)purin-9-yl)-methyloxypentane (42a, 795 mg, 1.725 mmol) in methanol (50 ml) was saturated at 0°C with ammonia, and the mixture heated in a sealed glass tube at 110°C for 20 hours. The tube was then cooled to 0°C, its contents transferred to a flask, and the solvent evaporated. The residue was chromatographed on silica gel (CH_2Cl_2/methanol 9:1 as eluant) to yield 1'-pivaloyloxy-5'-benzyloxy-3'-(aden-9-yl)-methyloxypentane (43a, 600 mg, 79%) as a colorless oil.

1'-Pivaloyloxy-5'-benzyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentane 44a

To a suspension of 1'-pivaloyloxy-5'-benzyloxy-3'-(aden-9-yl)-methyloxypentane (43a, 390 mg) in dry pyridine (20 ml) was added trimethylchlorosilane (5 fold excess), and the mixture stirred for 15 min. Benzoyl chloride (1 ml) was then added, and the mixture stirred for 2.5 hours. The mixture was then cooled on an ice bath, and diluted with water (10 ml). After 5 min, 29% aqueous ammonia (10 ml) was added, and the mixture stored at room temperature for 30 min. The reaction mixture was then evaporated to near dryness, and the residue dissolved in saturated sodium bicarbonate solution (15 ml), and the solution extracted three times with methylene chloride. The extracts were dried (magnesium sulfate) and flashed with hexane:EtOAc (1:1) as eluant to yield 1'-pivaloyloxy-5'-benzyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentane (44a, 305 mg, 56%).

5'-Pivaloyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentan-1-ol

45a

1'-Pivaloyloxy-5'-benzyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentane (44a, 380 mg, 0.456 mmol) is dissolved in a cyclohexene:ethanol (3:10) mixture, palladium hydroxide (300 mg, 20% on carbon) is added, and the reaction mixture is heated at 80°C overnight. The reaction mixture is filtered through Celite, the Celite is washed with ethanol, and the combined filtrates is evaporated to yield an oil. The oil is purified by flash chromatography on silica gel to yield 5'-pivaloyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentan-1-ol (45a).

5'-Pivaloyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentan-1-thiol S-acetate 46a

DEAD (0.03 ml, 0.18 mmol) was added at 0°C to a solution of triphenylphosphine (47 mg, 0.18 mmol) in THF (0.5 ml). The mixture is stirred for 30 min. 5'-Pivaloyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentan-1-ol (45a, 0.09 mmol) in THF (0.5 ml) and thioacetic acid (0.013 ml, 0.18 mmol)) in THF (0.5 ml) are then added to the solution. The reaction mixture is stirred at 0°C for 1 hour, and then for another hour at RT. The solvents are then evaporated under vacuum, and the residue chromatographed on silica gel (20 g, EtOAc eluant) to yield 5'-pivaloyloxy-3'-(6-N-benzoyladen-9-yl)-methyloxypentan-1-thiol S-acetate (46a).

This compound could be conveniently stored, and hydrolyzed in base to the free thiol-alcohol building block (47a) immediately before coupling.

Example 6 (Figure 10)

Non-rigid, Isosteric, Heteroatom in Linker

The uracil analog was prepared analogously. The chloromethyl ether (41, 3.6 g)) was added to a refluxing mixture of CH_2Cl_2 (20 ml), tetrabutylammonium iodide (13.5 mg) and 2.5 ml of 2,4-bis(trimethylsilyloxy)pyrimidine was refluxed for 1 hour. The reaction mixture was diluted with methanol, the solvents removed, and the product purified by flash chromatography to yield 1'-pivaloyloxy-5'-benzyloxy-3'-(urid-3-yl)methyloxypentane (43b, 1.04 g).

1'-Pivaloyloxy-5'-benzyloxy-3'-(urid-3-yl)methyloxypentane (43b, 710 mg) dissolved in a mixture of ethanol:cyclohexene (28 ml, 5:2) and treated with palladium oxide (400 mg) as described above yielded 5'-pivaloyloxy-3'-(urid-3-yl)methyloxypentan-1-ol (45b). To a solution of this product (45b, 100 mg, 0.304 mmol) in THF (2 ml) was added thioacetic acid (30 mg), triphenylphosphine 111 mg, and DEAD 80 ul using the procedure described above to yield 5'-pivaloyloxy-3'-(uridyl-1-yl)-methyloxypentan-1-thiol S-acetate (46b, 91 mg, 77%).

As above, this compound could be conveniently stored, and hydrolyzed in base to the free thiol-alcohol building block (47b) immediately before coupling.

Example 7 (Figure 11)

Non-rigid, Not Isosteric, No Heteroatom in Linker, Class (0,1,1)

2-methyl-3-ethoxyacryloyl isocyanate 50

Silver isocyanate (4.166 g, 27.8 mmol, dried in the dark over phosphorus pentoxide at 135 °C) was suspended in anhydrous benzene (20 ml). To the suspension was added 2-methyl-3-ethoxyacryloyl chloride (2.369 g, 16 mmol)\(18) in benzene (15 ml). The mixture was heated at reflux for a half hour, and then cooled slowly to RT over a period of 2.5 hours. This procedure yielded a solution of 2-methyl-3-ethoxyacryloyl isocyanate 50 in benzene.

Disulfide of ethyl 2'-(uridyl-1-yl)-3-mercaptopropionate 52

In a separate flask, the dimethylester of L-cystine (51, commercial as hydrochloride, 1.703 g, 5.0 mmol), triethylamine (1.478 g, 14.6 mmol), and pyrrolidinopyridine (319 mg, 2.2 mmol), was dissolved in dimethylformamide (30 ml). The solution of 2-methyl-3-ethoxyacryloyl isocyanate 50 in benzene prepared above was then added to this solution at -12°C over a period of 45 minutes, and the mixture was stirred at RT for an additional 12 hours. The solvents were then removed under vacuum (0.01 torr), and the residue chromatographed over Kieselgel (CH_2Cl_2-ethanol as eluant) to yield 3.24 g of an amide intermediate.

The amide intermediate (6.584 g, 11.37 mmol) was dissolved in ethanol (40 ml) and 2 N sulfuric acid (40 ml), and the mixture refluxed for 5 hours. Most of the solvent was removed at reduced pressure, the residue was suspended in ethanol (30 ml) and benzene (200 ml), and the water removed by azeotropic distillation. The solvents were removed, and

dissolved in CH_2Cl_2. The organic solution was extracted with saturated aqueous sodium bicarbonate, saturated aqueous sodium chloride, and dried. The disulfide of ethyl 2'-(uridyl-1-yl)-3-mercaptopropionate (52, 5.966 g) was isolated following removal of the solvent.

The pyrimidine ring was protected by an N-mesityl group \(19) through reaction with the hexachloroantimoniate salt of the mesityl cation in CH_2Cl_2 with pyridine (2 equivalents) to yield a suitably protected building block precursor (53). This compound could be conveniently stored, and the free thiol-alcohol building block (54) produced immediately prior to coupling by reduction with lithium triethylborohydride using the following procedure.

Building Block 54

To a solution under argon of disulfide 53 (1.088 g, 2.1 mmol) in THF (10 ml) at -18°C was added 20 ml of a 1 M solution of lithium triethylborohydride in THF. The reaction mixture was stirred at -18°C for 30 minutes, and then allowed to warm to RT. After 24 hours, 10 ml of a saturated aqueous solution of ammonium chloride was added, and the building block (54) purified by chromatography on Kieselgel (CH_2Cl_2-ethanol as eluant).

Example 8 (Figure 12)

Non-rigid, Not Isosteric, No Heteroatom in Linker, Class (0,1,2)

Disulfide of N-(2-amino-4-chloropurin-9-yl)-homocysteine 60

A mixture of 2-amino-4,6-dichloropyrimidine (54, commercial, > 99%, 13.6 g, 83 mmol, 3 fold excess), the dimethyl ester of homocystine (55, commercial, 27 mmol), triethylamine (10 ml) (350 ml) is refluxed in ethanol for 24 hours. The reaction mixture is then placed in a freezer, where unreacted 2-amino-4,6-dichloropyrimidine slowly separates from the cold mixture and is removed by filtration. The filtrate is concentrated under reduced pressure to yield a partially solid residue.

4-Chlorobenzene diazonium chloride (57) is prepared at 0°C from 10.6 g (83 mmol) 4-chloroaniline, 23 ml HCl (12 N), and sodium nitrite (6.3 g, 90 mmol) in 78 ml water.\(20) The cold solution of the diazonium salt is added dropwise over a period of 30 min to a stirred solution (at RT) of product of previous reaction, sodium acetate trihydrate (150 g), acetic acid (375 ml). and water (400 ml). The mixture is stirred overnight at RT. Diazo intermediate 58 is collected as a precipitate by filtration.

The diazo compound (58, 14 mmol) is suspended in ethanol (120 ml), water (120 ml) and acetic acid (12 ml) under nitrogen at 70°C. Zinc dust (11.5 g) is added slowly (over 1 hour). Heating is continued for another 1.5 hours. The mixture is filtered under nitrogen, the precipitate washed with ethanol, the filtrate and washings combined and concentrated to 0.25 of the original volume under vacuum. 4-Chloroaniline is removed from the aqueous mixture by extraction with ether, the pH raised to 6.0 with NaOH (2 N), and triamine 59 is collected as a precipitate, washed (cold

water) and dried. To the triamine (59, 2.5 mmol) in cold dimethylacetamide (6 ml) is added triethylorthoformate (5.2 ml, freshly distilled) and HCl (12 N, 0.25 ml). The mixture is stirred overnight under nitrogen at RT, concentrated under vacuum, dissolved in 88% formic acid (15 ml), refluxed overnight, and concentrated in vacuum. Remaining solvent is removed by several evaporations of methanol. The disulfide of N-(2-amino-4-chloropurin-9-yl)-homocysteine 60 is then precipitated from cold ammonia-methanol (10% ammonia).

This product could be converted to the protected precursor of the guanosine analog building block 61 by acidic hydrolysis,\(20) followed by benzoylation by the procedure described above. Product 61 could be conveniently stored, and converted to the thiol-alcohol building block (62) immediately before coupling by reduction with lithium borohydride.

Example 9 (Figure 13)

Non-rigid, Not Isosteric, No Heteroatom in Linker, Class (1,1,1)

2-(N-Benzoyl-adenyl-9-yl)methyl-3-mercaptopropanol 70

A solution of triphenylphosphine (730 mg, 2.8 mmol) and 6-chloropurine (430 mg, 2.8 mmol) in dry THF (16 ml) is added to a solution of DEAD (0.42 ml) in dry THF (3.5 ml). The mixture was stirred for 2 hours at RT. To the solution is added enantiomerically pure 2(R)-benzyloxymethyl-3-(tetrahydropyran-2-yloxy)-1-propanol (64, 1.9 mmol)\(21) in THF (9 ml). After the reaction is complete, the solvents were

evaporated, and the residue containing intermediate 65 purified by chromatography.

The intermediate 65 is dissolved in methanol saturated at 0°C with ammonia, and the mixture heated in a sealed glass tube at 110°C for 20 hours. The tube is then cooled to 0°C, its contents transferred to a flask, and the solvent evaporated. The residue is chromatographed on silica gel to yield 9-((2(R)-benzyloxymethyl-3-tetrahydropyran-2-yloxy)-propan-1-yl)-adenine (66). To a suspension of 66 (400 mg) in dry pyridine (20 ml) is added trimethylchlorosilane (5 fold excess), and the mixture stirred for 15 min. Benzoyl chloride (1 ml) was then added, and the mixture stirred for 2.5 hours. The mixture is then cooled on an ice bath, and diluted with water (10 ml). After 5 min, 29% aqueous ammonia (10 ml) is added, and the mixture stored at room temperature for 30 min. The reaction mixture is then evaporated to near dryness, the residue dissolved in saturated sodium bicarbonate solution (15 ml), and the solution extracted three times with methylene chloride. The extracts are dried (magnesium sulfate) and flashed with hexane:EtOAc (1:1) as eluant to yield N-benzoyl 9-((2(R)-benzyloxymethyl-3-tetrahydropyran-2-yloxy)-propan-1-yl)-adenine 67).

Intermediate 67 (0.456 mmol) is dissolved in a cyclohexene:ethanol (3:10) mixture together with palladium hydroxide (300 mg, 20% on carbon). The reaction mixture is heated at 80°C overnight, then filtered through Celite. The Celite is washed with ethanol, and the combined filtrates were evaporated to yield an oil. The oil is purified by flash

chromatography on silica gel to yield N-benzoyl 9-((2(R)-hydroxymethyl-3-tetrahydropyran-2-yloxy)-propan-1-yl)-adenine 68.

DEAD (0.03 ml, 0.18 mmol) is added at 0°C to a solution of triphenylphosphine (47 mg, 0.18 mmol) in THF (0.5 ml). The mixture is stirred for 30 min. The product of the previous reaction (68, 0.09 mmol) in THF (0.5 ml) and thioacetic acid (0.013 ml, 0.18 mmol)) in THF (0.5 ml) are then added to the solution. The reaction mixture is stirred at 0°C for 1 hour, and then for another hour at RT. The solvents are then evaporated under vacuum, and the residue chromatographed on silica gel to yield N-benzoyl 9-((2(R)-acetylthiomethyl-3-tetrahydropyran-2-yloxy)-propan-1-yl)-adenine (69).

This compound is conveniently stored, and the free thiol-alcohol building block (70) prepared by hydrolysis of 69 in acid and then base immediately before coupling.

Example 10 (Figure 14)

Non-rigid, Not Isosteric, No Heteroatom in Linker, Class (1,2,1)

2-(N-Benzoyl-guanin-9-yl)methyl-3-mercaptopropanol 78

Enantiomerically pure 2(R)-benzyloxymethyl-4-(tetrahydropyran-2-yloxy)-1-butanol (71, 1.9 mmol) \(22) in dimethylformamide is added to a solution of triphenylphosphine and carbon tetrabromide in dimethylformamide to yield 1-bromo-2(R)-benzyloxymethyl-4-(tetrahydropyran-2-yloxy)-1-butane (72. To a solution of 72 in dimethylformamide is added 2-amino-6-

chloropurine. After stirring at room temperature (8 hours), 9-((2(R)-benzyloxymethyl-4-tetrahydropyran-2-yloxy)-butan-1-yl)-2-amino-6-chloropurine (73) is isolated and purified by chromatography on silica gel. Acid hydrolysis (2N HCl, reflux, 75 min) converts this compound to 9-((2(R)-benzyloxymethyl-4-hydroxy)-butan-1-yl)-guanine (74), which is purified by chromatography on silica gel.

To a suspension of 74 (300 mg) in dry pyridine (20 ml) is added trimethylchlorosilane (5 fold excess), and the mixture stirred for 15 min. Benzoyl chloride (1 ml) was then added, and the mixture stirred for 2.5 hours. The mixture is then cooled on an ice bath, and diluted with water (10 ml). After 5 min, 29% aqueous ammonia (10 ml) is added, and the mixture stored at room temperature for 30 min. The reaction mixture is then evaporated to near dryness, the residue dissolved in saturated sodium bicarbonate solution (15 ml), and the solution extracted three times with methylene chloride. The extracts are dried (magnesium sulfate) and flashed with hexane:EtOAc (1:1) as eluant to yield N-benzoyl 9-((2(R)-benzyloxymethyl-4-benzoyloxy)-butan-1-yl)-guanine (75), which is purified by chromatography on silica gel.

Following the removal of the benzyl protecting group by catalytic reduction as described previously to yield intermediate 76, and conversion of the free hydoxyl group to an acetylthio group as described previously, the S-acetate of 9-((2(R)-mercaptomethyl-4-benzoyloxy)-butan-1-yl)-guanine (77 is isolated. This compound could be conveniently stored, and the free thiol-hydroxy building block (78) obtained by mild

alkaline hydrolysis immediately prior to condensation.

Example 11 (Figure 15)

Non-rigid, Not Isosteric, No Heteroatom in Linker, Classes (2,2,2) and (2,1,3)

1-R-Norbornanone 81

Pyridinium chlorochromate (23 g, 107 mmol) is stirred with Celite (22 g) and CH_2Cl_2 (140 ml) at RT. To the suspension is added (1S,2S)-norbornan-2-ol (80, 70 mmol)\(23) in CH_2Cl_2 (70 ml) dropwise over a period of 30 min. The mixture is stirred for an hour at RT, at which point the dark brown suspension is filtered through silica gel (60 g). Chromatography yields (1R)-norbornanone 81.

This product is oxidized by the Baeyer-Villiger procedure. (1R)-Norbornanone (81, 18 mmol) is stirred with sodium acetate (9 g, water-free), hydrogen peroxide (27 ml, 30%), and acetic acid (9 ml) at 5°C in the dark for 40 hours. The reaction is quenched by the addition of sodium sulfite (50 ml of a 10% aqueous solution) at 0°C. The product is extracted twice with EtOAc (600 ml). The extract is washed with the sodium sulfite solution, then with sodium bicarbonate solution (50 ml, saturated), then with sodium chloride solution (saturated), dried and evaporated. The product is chromatographed on silica (380 g, ether) to yield the lactone of 3-hydroxycyclopentane-1-acetic acid (82, 12 mmol, 67%).

This product is hydrolyzed in base, oxidized to 3-carboxymethylcyclopentanone (83) with chromic acid (vide

supra), and oxidized again using a Baeyer-Villager procedure (vide supra) to yield a mixture of (4S)-2-oxa-4-carboxymethyl-tetrahydropyran (84) and (5S)-2-oxa-5-carboxymethyl-tetrahydropyran (85) These compounds are separated, and serve as the skeletons for synthesis of flexible building blocks in Class (2,2,2) and (2,3,1), exemplified by structures 86 and 87, following the sequence of reactions shown in Figure 15, using procedures analogous to those described in Examples 1-10.

Figure 1 shows structures that illustrate the terms "growing oligonucleotide chain", building block", "coupling reaction", "building block", "oligomer subunit", and "oligonucleotide analog". Figure 2 shows rigid isosteric building blocks with and without heteroatoms, together with a short oligonucleotide built from them designed to bind to a natural oligonucleotide with the sequence GAC. Figure 3 shows flexible isosteric building blocks with and without heteroatoms, together with a short oligonucleotide built from them designed to bind to a natural oligonucleotide with the sequence GAC. Figure 4 list flexible non-isosteric building blocks listed by class, where the three numbers indicate (consecutively) the number of linking atoms in L, X, and Y in Formula 1; superscripts, where present, indicate the presence of linking heteroatoms in these groups. These building blocks can be synthesized from compounds shown to their immediate right that are either commercially available or known in the prior art (see references 24-29). Figure 5 lists examples of suitable base analogs. These compounds are known in the prior art, as are methods that can be readily adapted to incorporate them into the building blocks disclosed here (see references 30-36).

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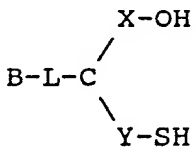
What is claimed is:

1. Compositions of matter with the formula:



in which B (a "base analog") is a heterocyclic ring system selected from the group consisting of pyrimidine, azapyrimidine, purine, azapurine, pyrrolopyrimidine, pyrazolopyrimidine, triazolopyrimidine, imidazolopyridine, pyrrolopyridine, pyrazolopyridine, and triazolopyridine, and where the ring may be functionalized with amino groups, hydroxyl groups, halogen groups or acylated derivatives of amino or hydroxyl groups; L is a linking group containing from 0 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-; C is a carbon atom with defined chirality; X is a linking group containing from 1 to 5 linking units selected from the group consisting of $-CH_2-$ and -O-; Y is a linking group containing from 0 to 5 linking units selected from the group consisting of $-CH_2-$ and -O-; OH is a hydroxyl group; and SH is a thiol group.

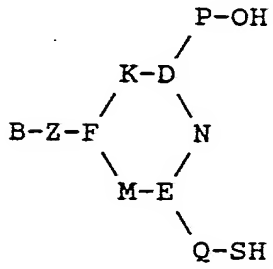
2. Compositions of claim 1, in which X is selected from the group consisting of $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; Y is selected from the group (-) (i.e., bond), $(-CH_2-)$, $(-CH_2CH_2-)$ $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; L is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$ (CH_2O-), $(-CH_2CH_2CH_2-)$, and $(-CH_2CH_2O-)$.

3. Compositions of claim 2, in which B is selected from the

group, adenine, guanine, 2,6-diaminopurine, 3-deazaguanine, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-8-azaguanine, 3,7-dideazaguanine, 3,7-dideaza-8-azaguanine, 7-deazaguanine, 3,7-dideazaadenine, hypoxanthine, 8-deazaadenine, 8-azaguanine, 5-azauracil, uracil, thymine, cytosine, 6-azauracil, 6-azathymine, 6-azacytosine, 5-fluorouracil, 5-bromouracil, 5-iodouracil, 5-azauracil, and 5-azacytosine.

4. Compositions of claim 3, in which B is selected from the group adenine, guanine, cytosine, thymine, and uracil.

5. Compositions of matter with the formula:



in which B (a "base analog") is a heterocyclic ring system selected from the group consisting of pyrimidine, azapyrimidine, purine, azapurine, pyrrolopyrimidine, pyrazolopyrimidine, triazolopyrimidine, imidazolopyridine, pyrrolopyridine, pyrazolopyridine, and triazolopyridine, and where the ring may be functionalized with amino groups, hydroxyl groups, halogen groups or acylated derivatives of amino or hydroxyl groups; K, M and N are linking groups containing from 0 to 3 linking units selected from the group consisting of $-CH_2-$ and -O-, with the sum of linking units

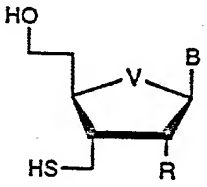
in K, M, and N less than 5; D, E, and F are CH groups with defined chiralities at carbon; P is a linking group containing from 1 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-, and Q and Z are linking groups containing from 0 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-, with the sum of linking units in P, Z, and Q less than 6; OH is a hydroxyl group; and SH is a thiol group.

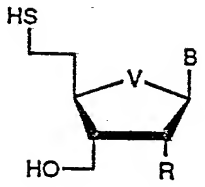
6. Compositions of claim 5, in which P is selected from the group $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; Q is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; Z is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$ $(-O-CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; and D, E, and F are CH groups with defined chirality.

7. Compositions of claim 6, in which B is selected from the group, adenine, guanine, 2,6-diaminopurine, 3-deazaguanine, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-8-azaguanine, 3,7-dideazaguanine, 3,7-dideaza-8-azaguanine, 7-deazaguanine, 3,7-dideazaadenine, hypoxanthine, 8-deazaadenine, 8-azaguanine, 5-azauracil, uracil, thymine, cytosine, 6-azauracil, 6-azathymine, 6-azacytosine, 5-fluorouracil, 5-bromouracil, 5-iodouracil, 5-azauracil, and 5-azacytosine.

8. Compositions of claim 7, in which B is selected from the group adenine, guanine, cytosine, thymine, and uracil.

9. Compositions of claim 8, in which D, E, F, K, M, N, P, Q, and Z are defined by the formula:



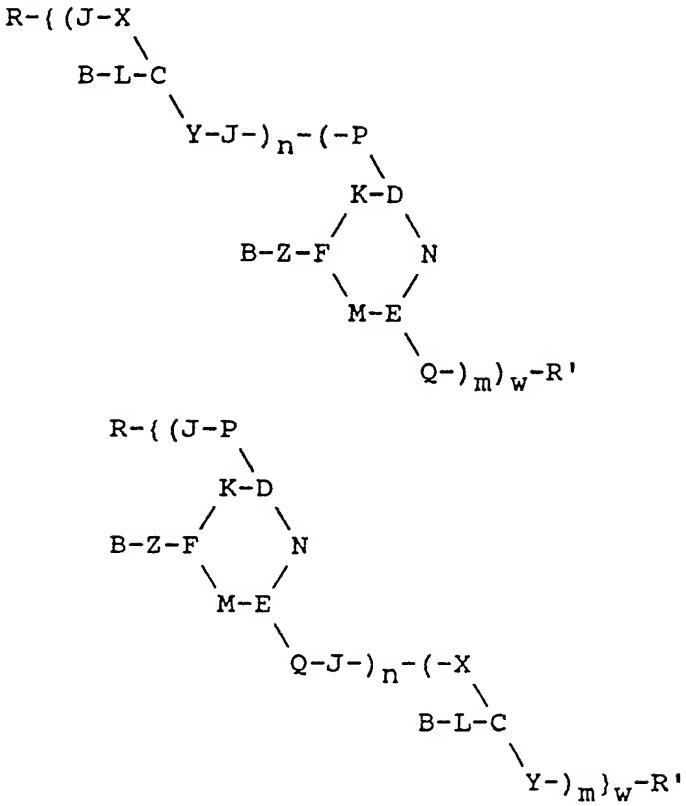


where T is selected from the group -O- or $-CH_2-$; R is selected from the group -H or -OH: and B (a "base analog") is a heterocyclic ring chosen from the group pyrimidine, azapyrimidine, purine, azapurine, imidazolopyrimidine, pyrrolopyrimidine, pyrazolopyrimidine, pyrrolopyridine, pyrazolopyridine, triazolopyrimidine, and triazolopyridine, and where the ring system may be functionalized with amino groups, hydroxyl groups, halogen groups or their protected derivatives.

10. Compositions of matter that are oligonucleotide analogs comprised of a set of base analogs joined in a defined sequence via linking groups stable to both enzymatic and chemical hydrolysis.

11. Compositions of claim 10 wherein said linking groups contain sulfide, sulfoxide, or sulfone units.

12. Compositions of claim 10 wherein ~~said~~ said oligonucleotide analogs are formed from building blocks defined in claims 1 through 8 joined in a defined sequence, with the formulae:



where n, m, and w are integers with a sum less than 50, J is a linking moiety selected from the group sulfide (-S-), sulfoxide (-SO-), and sulfone ($-SO_2$); B (a "base analog") is a heterocyclic ring system selected from the group consisting of pyrimidine, azapyrimidine, purine, azapurine, pyrrolopyrimidine, pyrazolopyrimidine, triazolopyrimidine, imidazolopyridine, pyrrolopyridine, pyrazolopyridine, and triazolopyridine, and where the ring may be functionalized with amino groups, hydroxyl groups, halogen groups or acylated derivatives of amino or hydroxyl groups; L's are linking groups containing from 0 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-; C's are CH groups with defined chirality at carbon; X's are linking groups containing from 1 to 5 linking units selected from

the group consisting of $-CH_2-$ and -O-; Y's are linking groups containing from 0 to 5 linking units selected from the group consisting of $-CH_2-$ and -O-; OH is a hydroxyl group; and SH is a thiol group; K's, M's and N's are linking groups containing from 0 to 3 linking units selected from the group consisting of $-CH_2-$ and -O-, with the sum of linking units in K, M, and N less than 5; D's, E's, and F's are carbon atoms with defined chiralities; P's are linking groups containing from 1 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-, and the Z's and Q's are linking groups containing from 0 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-, with the sum of linking units in P, Z, and Q less than 6; and R and R' are end groups selected from the group alkyl, alkaryl, and hydroxy.

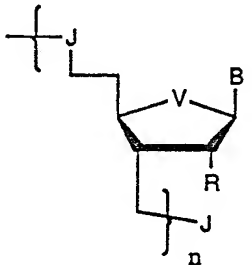
13. Compositions of claim 11, in which X is selected from the group (-) (i.e. bond), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-CH_2CH_2O-)$; Y is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; L is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2O-)$, $(-CH_2CH_2CH_2-)$, and $(-CH_2CH_2O-)$; P is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-CH_2CH_2O-)$; and Q is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2CH_2CH_2-)$, and $(-OCH_2CH_2-)$; Z is selected from the group (-), $(-CH_2-)$, $(-CH_2CH_2-)$, $(-CH_2O-)$, $(-CH_2CH_2CH_2-)$, and $(-CH_2CH_2O-)$.

14. Compositions of claim 13, in which B is selected from the

group, adenine, guanine, 2,6-diaminopurine, 3-deazaguanine, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-8-azaguanine, 3,7-dideazaguanine, 3,7-dideaza-8-azaguanine, 7-deazaguanine, 3,7-dideazaadenine, hypoxanthine, 8-deazaadenine, 8-azaguanine, 5-azauracil, uracil, thymine, cytosine, 6-azauracil, 6-azathymine, 6-azacytosine, 5-fluorouracil, 5-bromouracil, 5-iodouracil, 5-azauracil, and 5-azacytosine.

15. Compositions of claim 14, in which B is selected from the group consisting of adenine, guanine, cytosine, thymine, and uracil.

16. Compositions of claim 14, in which D, E, F, K, M, N, P, Q, and Z are defined by the formula:

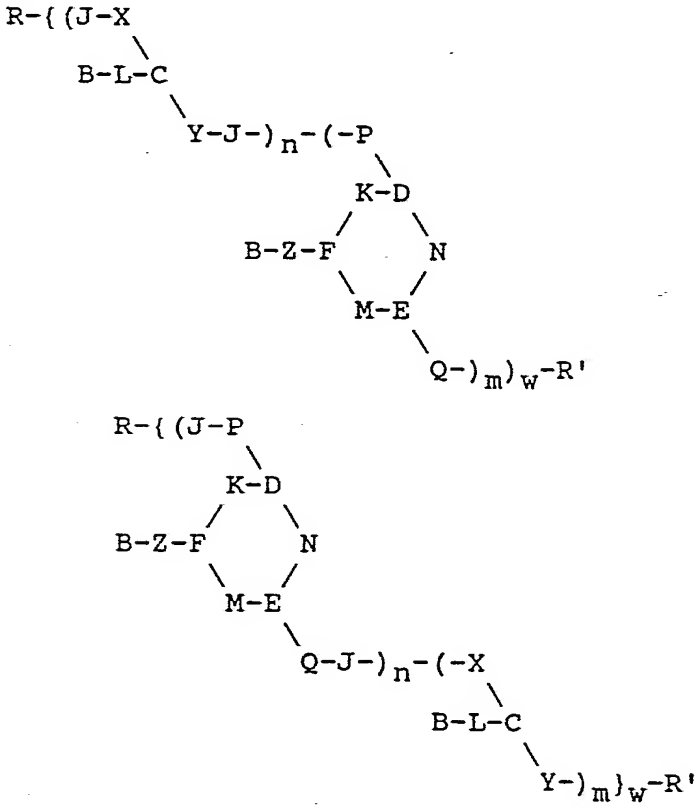


where T is selected from the group -O- or $-CH_2-$; R is selected from the group -H or -OH: and B (a "base analog") is a heterocyclic ring chosen from the group pyrimidine, azapyrimidine, purine, azapurine, imidazolopyrimidine, pyrrolopyrimidine, pyrazolopyrimidine, pyrrolopyridine, pyrazolopyridine, triazolopyrimidine, and triazolopyridine, and where the ring system may be functionalized with amino groups, hydroxyl groups, halogen groups or their protected derivatives.

17. A method for binding sequence-specifically to DNA or RNA oligonucleotides comprising the use of an oligonucleotide analog comprised of a set of base analogs joined in a defined sequence via linking groups stable to both enzymatic and chemical hydrolysis.

18. The method of claim 17 wherein said linking groups contain sulfide, sulfoxide, or sulfone units.

19. The method of claim 17, wherein said oligonucleotide analogs are formed from building blocks defined in claims 1 through 8 joined in a defined sequence, with the formulae:



where n, m, and w are integers with a sum less than 50, J is a linking moiety selected from the group sulfide (-S-), sulfoxide (-SO-), and sulfone ($-SO_2$); B (a "base analog") is a

heterocyclic ring system selected from the group consisting of pyrimidine, azapyrimidine, purine, azapurine, pyrrolopyrimidine, pyrazolopyrimidine, triazolopyrimidine, imidazolopyridine, pyrrolopyridine, pyrazolopyridine, and triazolopyridine, and where the ring may be functionalized with amino groups, hydroxyl groups, halogen groups or acylated derivatives of amino or hydroxyl groups; L's are linking groups containing from 0 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-; C's are CH groups with defined chirality at carbon; X's are linking groups containing from 1 to 5 linking units selected from the group consisting of $-CH_2-$ and -O-; Y's are linking groups containing from 0 to 5 linking units selected from the group consisting of $-CH_2-$ and -O-; OH is a hydroxyl group; and SH is a thiol group; K's, M's and N's are linking groups containing from 0 to 3 linking units selected from the group consisting of $-CH_2-$ and -O-, with the sum of linking units in K, M, and N less than 5; D's, E's, and F's are carbon atoms with defined chiralities; P's are linking groups containing from 1 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-, and the Z's and Q's are linking groups containing from 0 to 4 linking units selected from the group consisting of $-CH_2-$ and -O-, with the sum of linking units in P, Z, and Q less than 6; and R and R' are end groups selected from the group alkyl, alkaryl, and hydroxy.

20. The method of claim 19, in which X is selected from the

group (-) (i.e. bond), ($-CH_2-$), ($-CH_2CH_2-$), ($-CH_2CH_2CH_2-$), and ($-CH_2CH_2O-$); Y is selected from the group (-), ($-CH_2-$), ($-CH_2CH_2-$), ($-CH_2CH_2CH_2-$), and ($-OCH_2CH_2-$); L is selected from the group (-), ($-CH_2-$), ($-CH_2CH_2-$), ($-CH_2O-$), ($-CH_2CH_2CH_2-$), and ($-CH_2CH_2O-$); P is selected from the group (-), ($-CH_2-$), ($-CH_2CH_2-$), ($-CH_2CH_2CH_2-$), and ($-CH_2CH_2O-$); and Q is selected from the group (-), ($-CH_2-$), ($-CH_2CH_2-$), ($-CH_2CH_2CH_2-$), and ($-OCH_2CH_2-$); Z is selected from the group (-), ($-CH_2-$), ($-CH_2CH_2-$), ($-CH_2O-$), ($-CH_2CH_2CH_2-$), and ($-CH_2CH_2O-$).

21. The method of claim 20, in which B is selected from the group, adenine, guanine, 2,6-diaminopurine, 3-deazaguanine, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-8-azaguanine, 3,7-dideazaguanine, 3,7-dideaza-8-azaguanine, 7-deazaguanine, 3,7-dideazaadenine, hypoxanthine, 8-deazaadenine, 8-azaguanine, 5-azauracil, uracil, thymine, cytosine, 6-azauracil, 6-azathymine, 6-azacytosine, 5-fluorouracil, 5-bromouracil, 5-iodouracil, 5-azauracil, and 5-azacytosine.

22. Compositions of claim 21, in which B is selected from the group consisting of adenine, guanine, cytosine, thymine, and uracil.

23. The method of claim 19, in which D, E, F, K, M, N, P, Q, and Z are defined by the formula:

where T is selected from the group -O- or -CH_2-; R is selected from the group -H or -OH: and B (a "base analog") is a heterocyclic ring chosen from the group pyrimidine, azapyrimidine, purine, azapurine, imidazolopyrimidine, pyrrolopyrimidine, pyrazolopyrimidine, pyrrolopyridine, pyrazolopyridine, triazolopyrimidine, and triazolopyridine, and where the ring system may be functionalized with amino groups, hydroxyl groups, halogen groups or their protected derivatives.

Figure 1

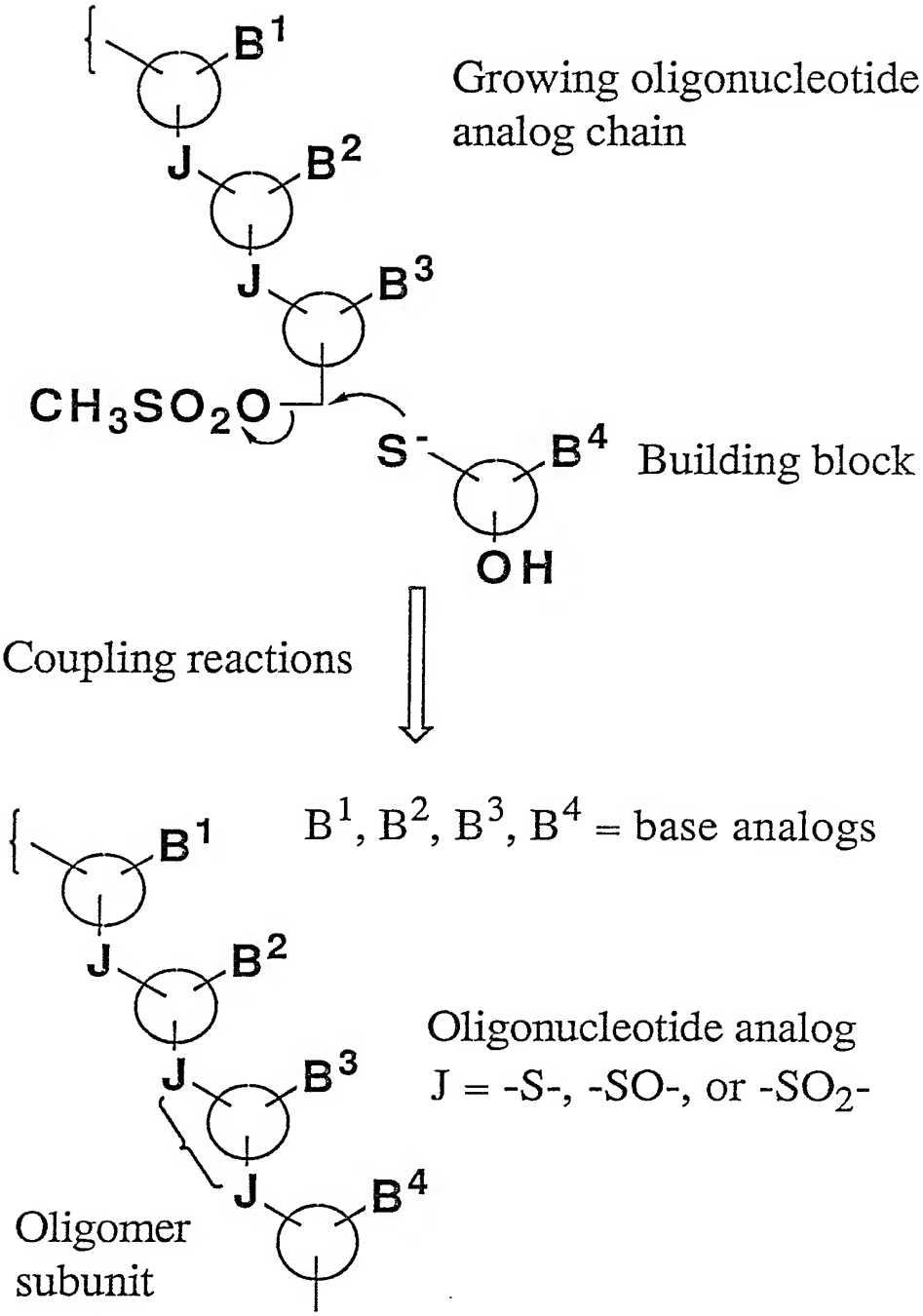

B1
J
B2
J
B3
CH3SO2O
S-
B4
OH
Growing oligonucleotide analog chain
Building block
Coupling reactions
B1, B2, B3, B4 = base analogs
Oligonucleotide analog
J = -S-, -SO-, or -SO2-
Oligomer subunit

Figure 2

Building Blocks

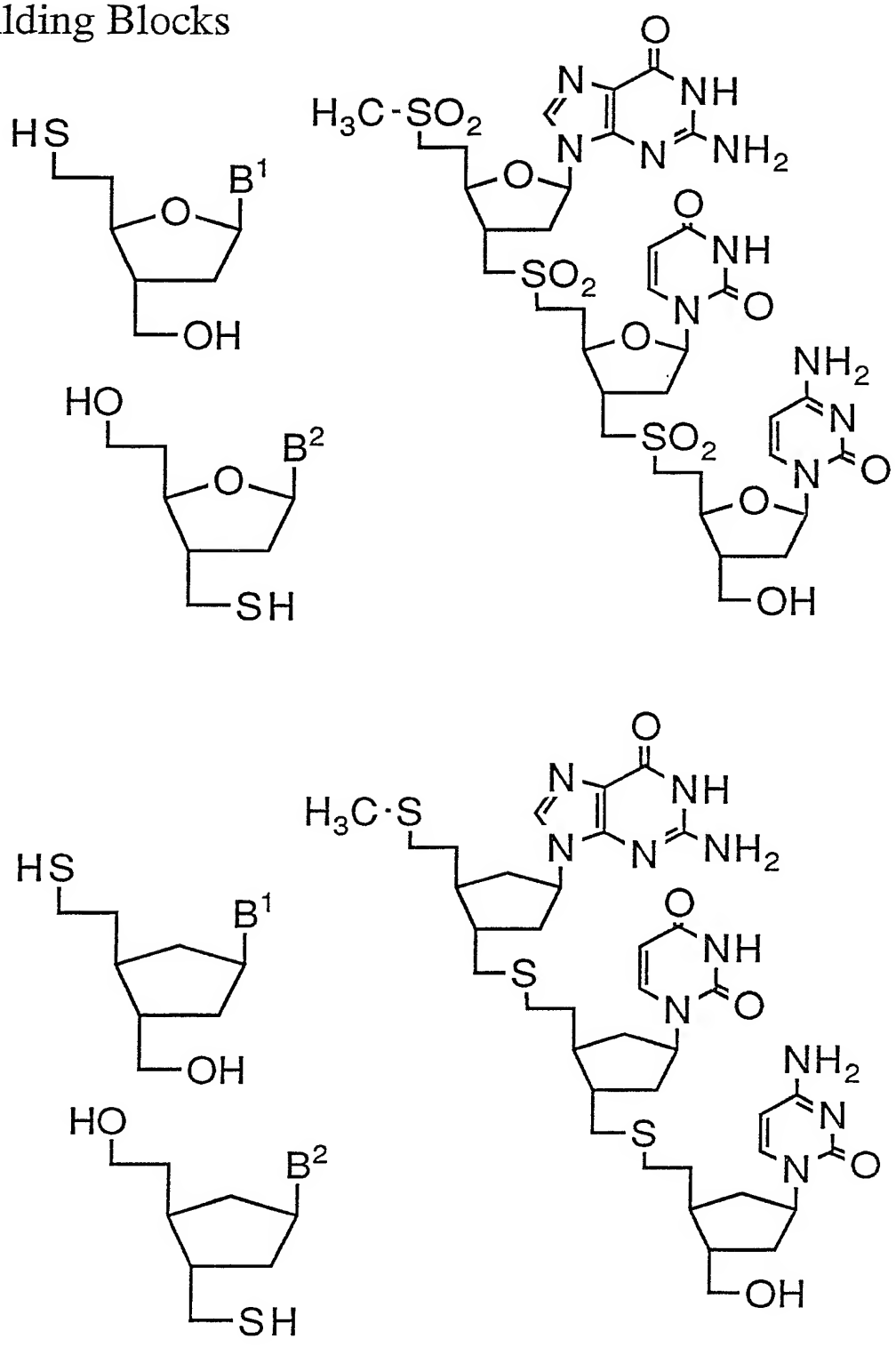
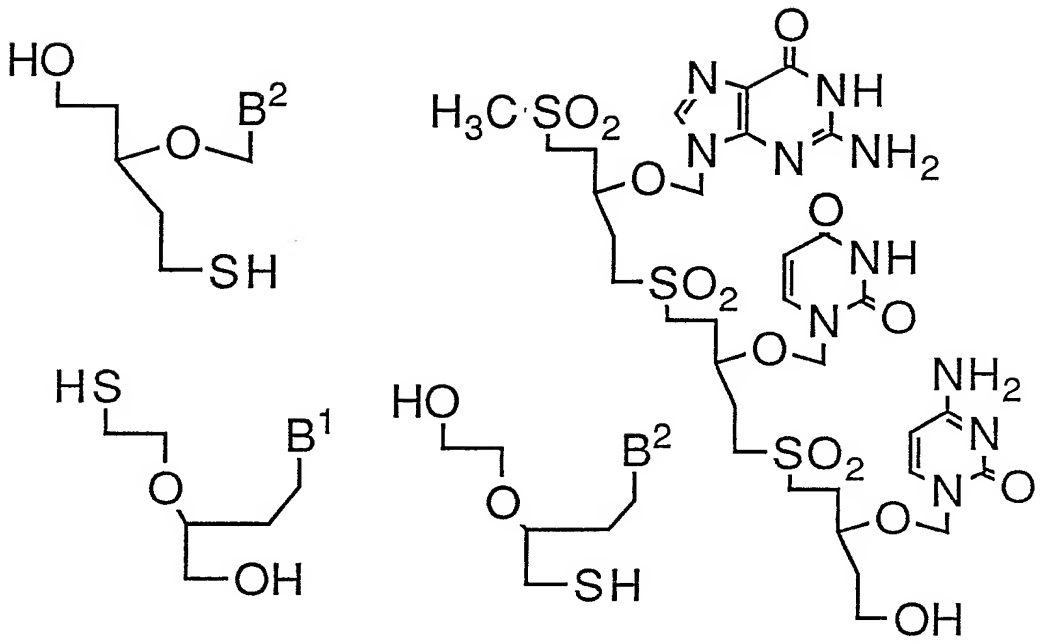


Figure 3


HO
B2
O
SH
HS
B1
O
OH
H3C·SO2
O
N
NH
N
N
NH2
SO2
O
NH
N
O
SO2
O
NH2
N
N
O
HO
B2
O
SH
OH

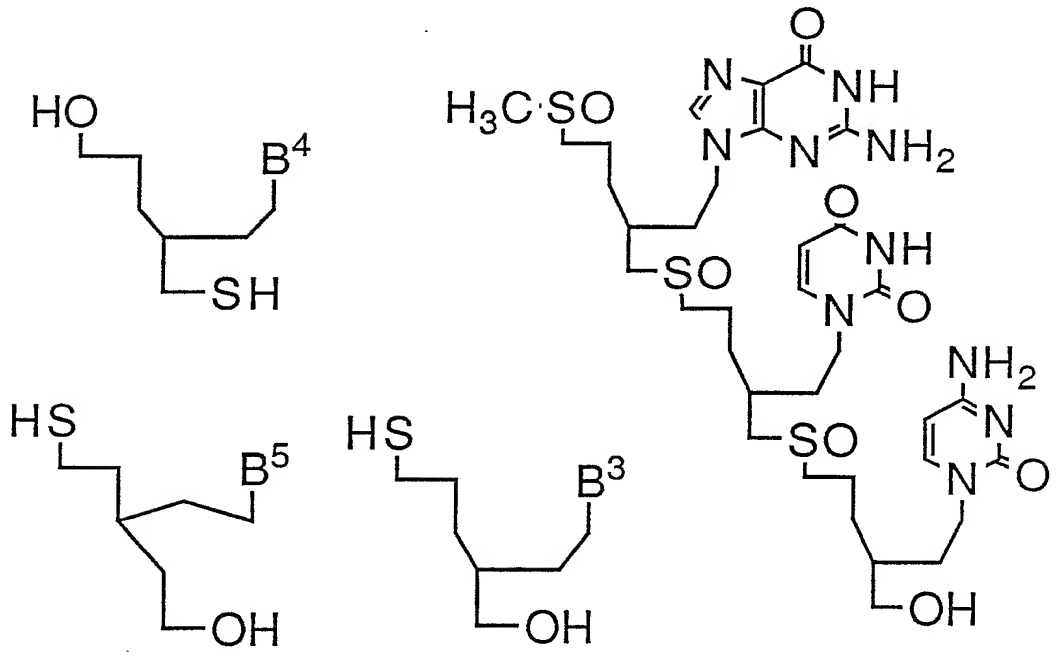
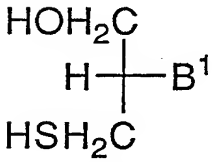
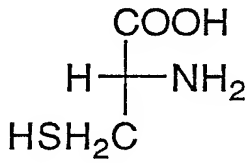
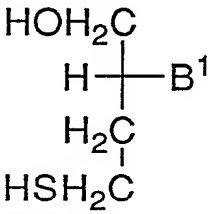
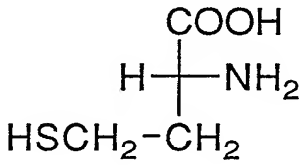
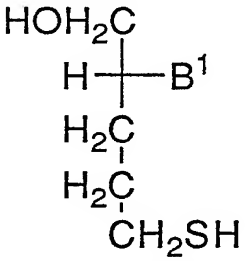
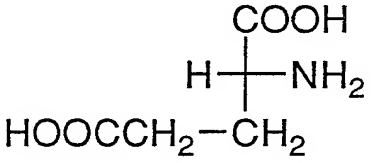
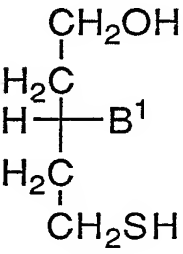
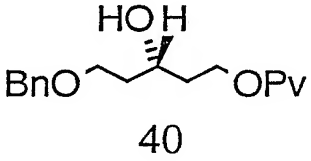
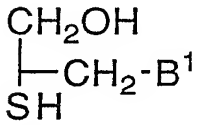
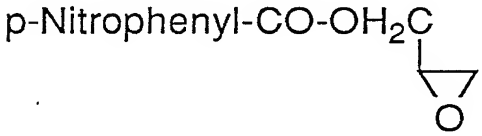
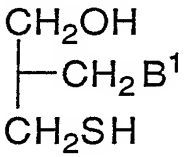
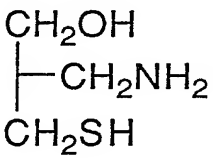
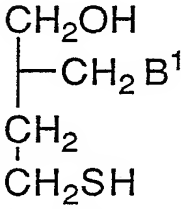
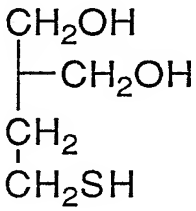

HO
B4
SH
HS
B5
OH
HS
B3
OH
H3C·SO
O
N
NH
N
N
NH2
SO
O
NH
N
O
SO
NH2
N
N
O
OH

Figure 4a

FLEXIBLE BUILDING BLOCKS

Class	Building Block	Skeleton from
0,1,1		
0,1,2		
0,1,3		
0,2,2		 40
1,1,0		
1,1,1		
1,1,2		

B^1=base analog

Figure 4b

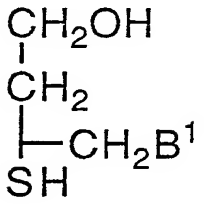
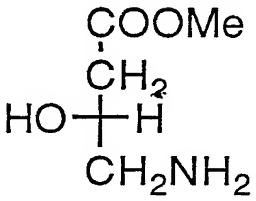
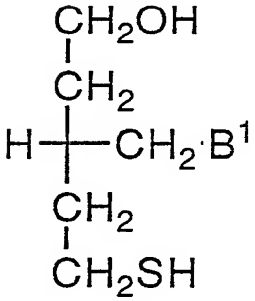
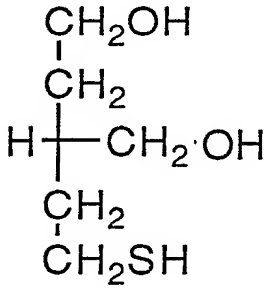
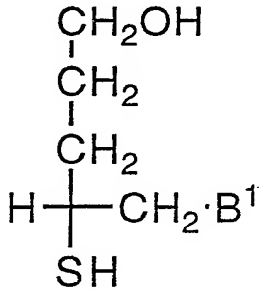
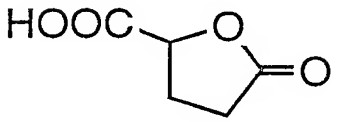
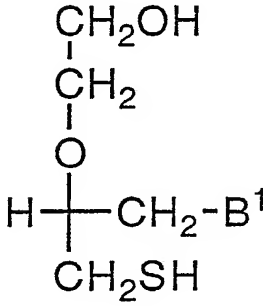
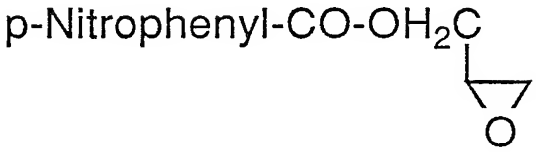
Class	Building Block	Skeleton from
1,2,0		
1,2,2		
1,3,0		
1,3^{O},1		

Figure 4c

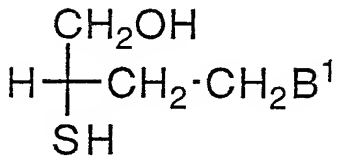
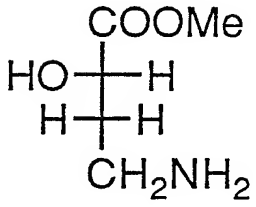
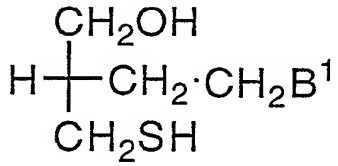
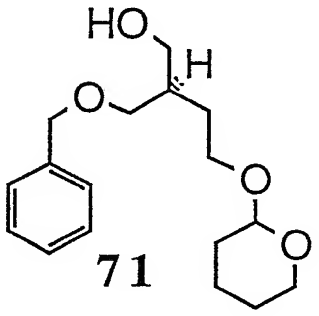
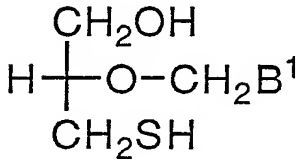
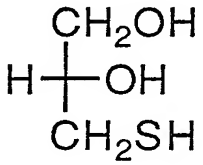
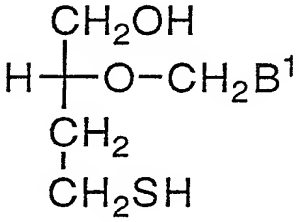
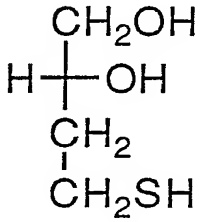
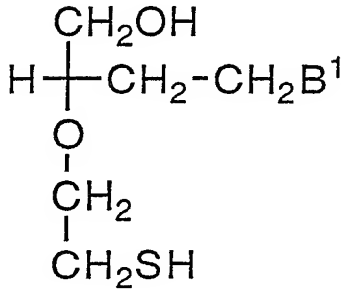
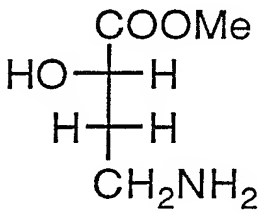
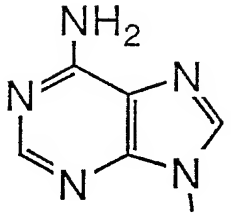
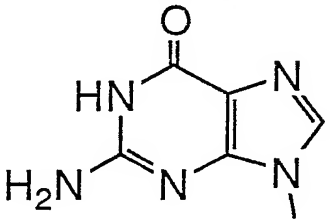
Class	Building Block	Skeleton from
2,1,0		
2,1,1		 71
2^{O},1,1		
2^{O},1,2		
2,1,3^{O}		

Figure 5a

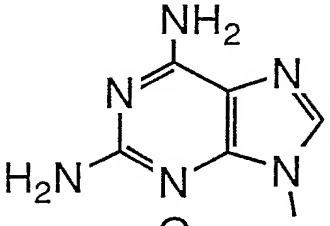
EXAMPLES OF BASE ANALOGS



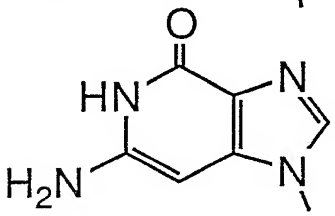
Adenine



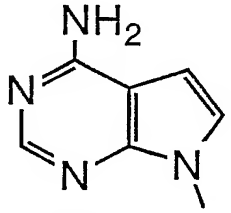
Guanine



Diaminopurine

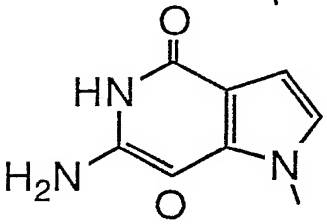


6-Amino-1,5-dihydroimidazo-[4,5-*c*]pyridin-4-one

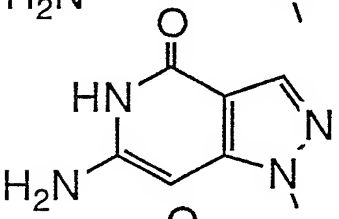


4-Aminopyrrolo-[2,3-*d*]pyrimidine (Tubercidin)

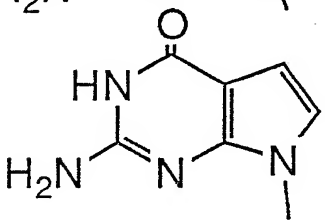
4-Aminopyrazolo-[3,4-*d*]pyrimidine



6-Amino-1H-pyrrolo-[3,2-*c*]-pyridin-4-one

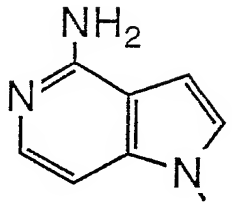


6-Amino-1H-pyrazolo-[4,3-*c*]pyridin-4-one

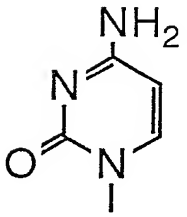


2-Amino-1H-pyrrolo-[2,3-*d*]pyrimidin-4-one

Figure 5b

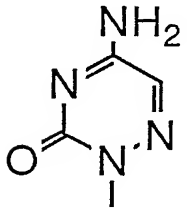


3,7-Dideaza-adenine

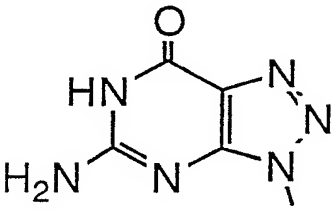


Cytosine

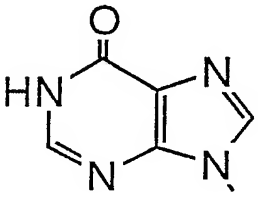
8-Azaadenine



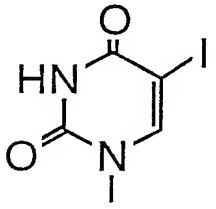
6-Azacytosine



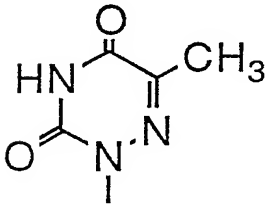
8-Azaguanine



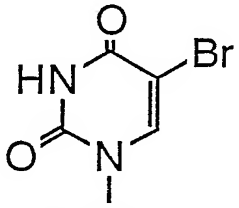
Hypoxanthine



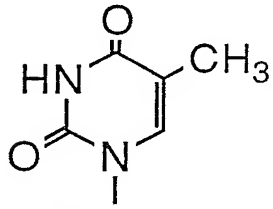
5-Iodouracil



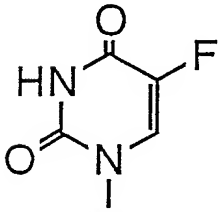
6-Azathymine



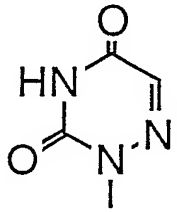
5-Bromouracil



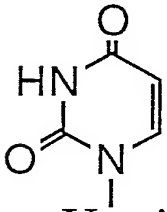
Thymine



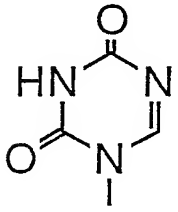
5-Fluorouracil



6-Azauracil



Uracil



5-Azauracil

Figure 6a

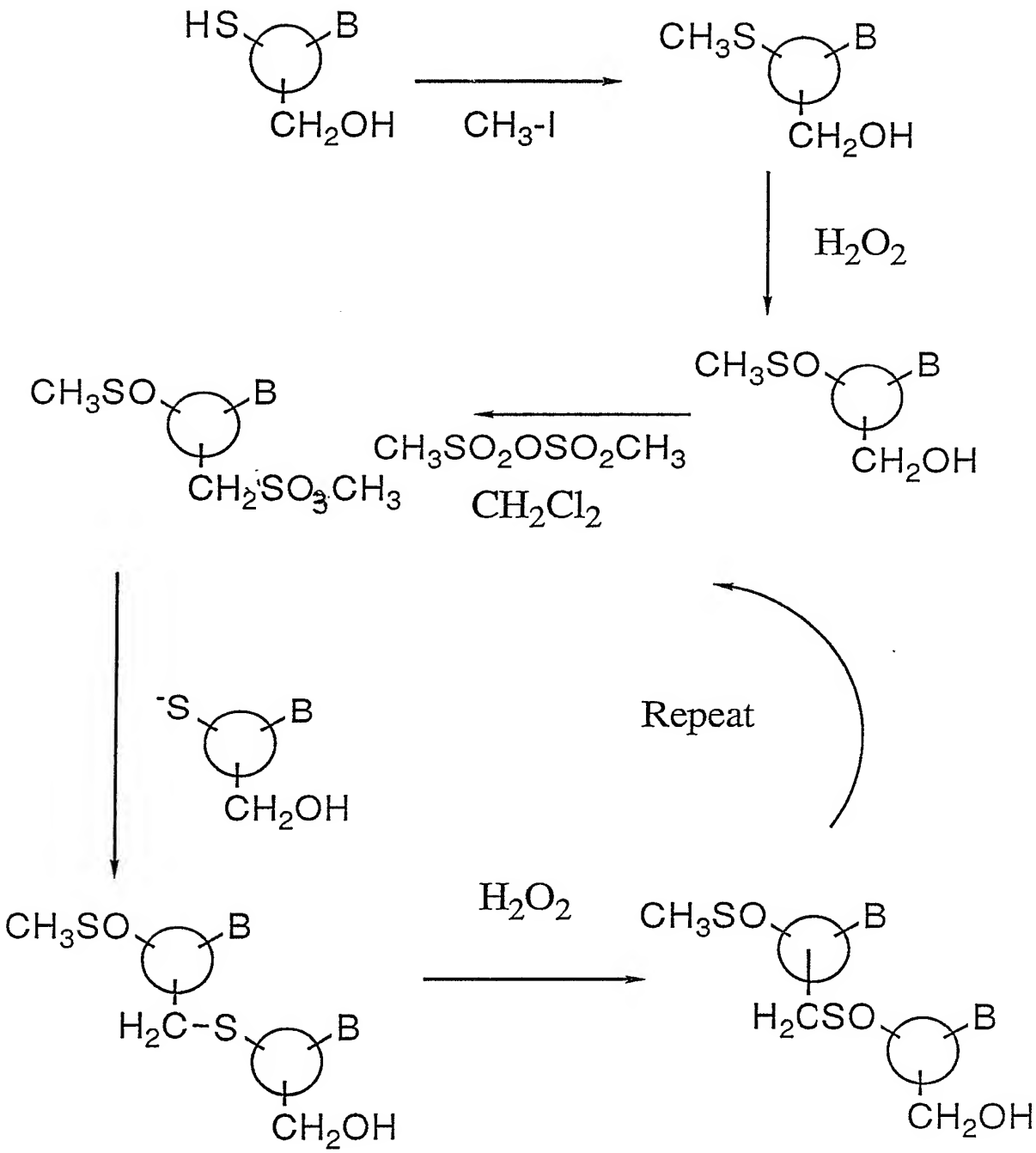

HS
B
CH2OH
CH3-I
CH3S
B
CH2OH
H2O2
CH3SO
B
CH2OH
CH3SO2OSO2CH3
CH2Cl2
CH3SO
B
CH2SO3CH3
-S
B
CH2OH
Repeat
H2O2
CH3SO
B
H2C-S
B
CH2OH
CH3SO
B
H2CSO
B
CH2OH

Figure 6b

EXAMPLE OF OLIGONUCLEOTIDE ANALOG BINDING TO A-C-C-T-C-C-T

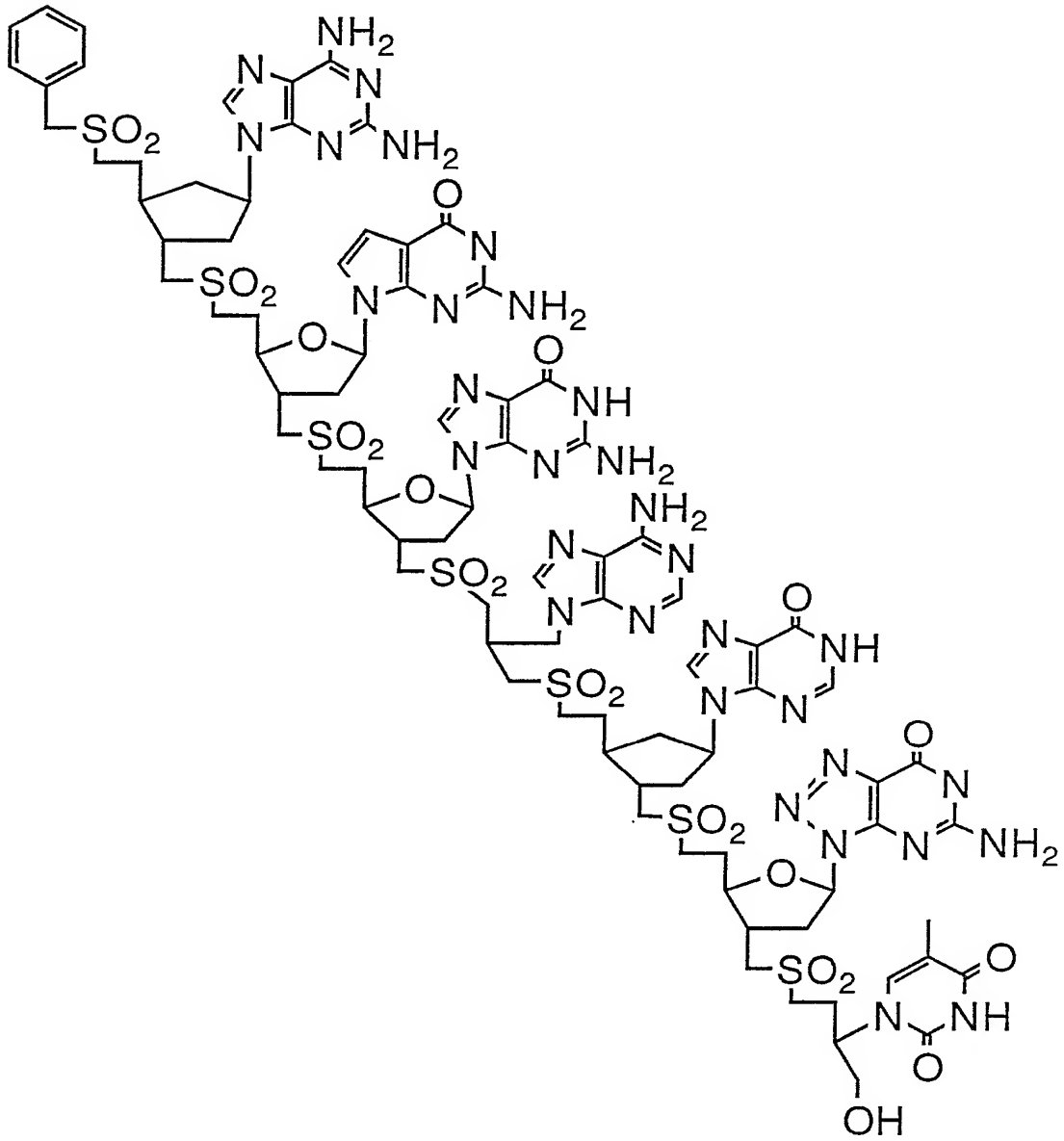


Figure 7 EXAMPLES 1 and 2

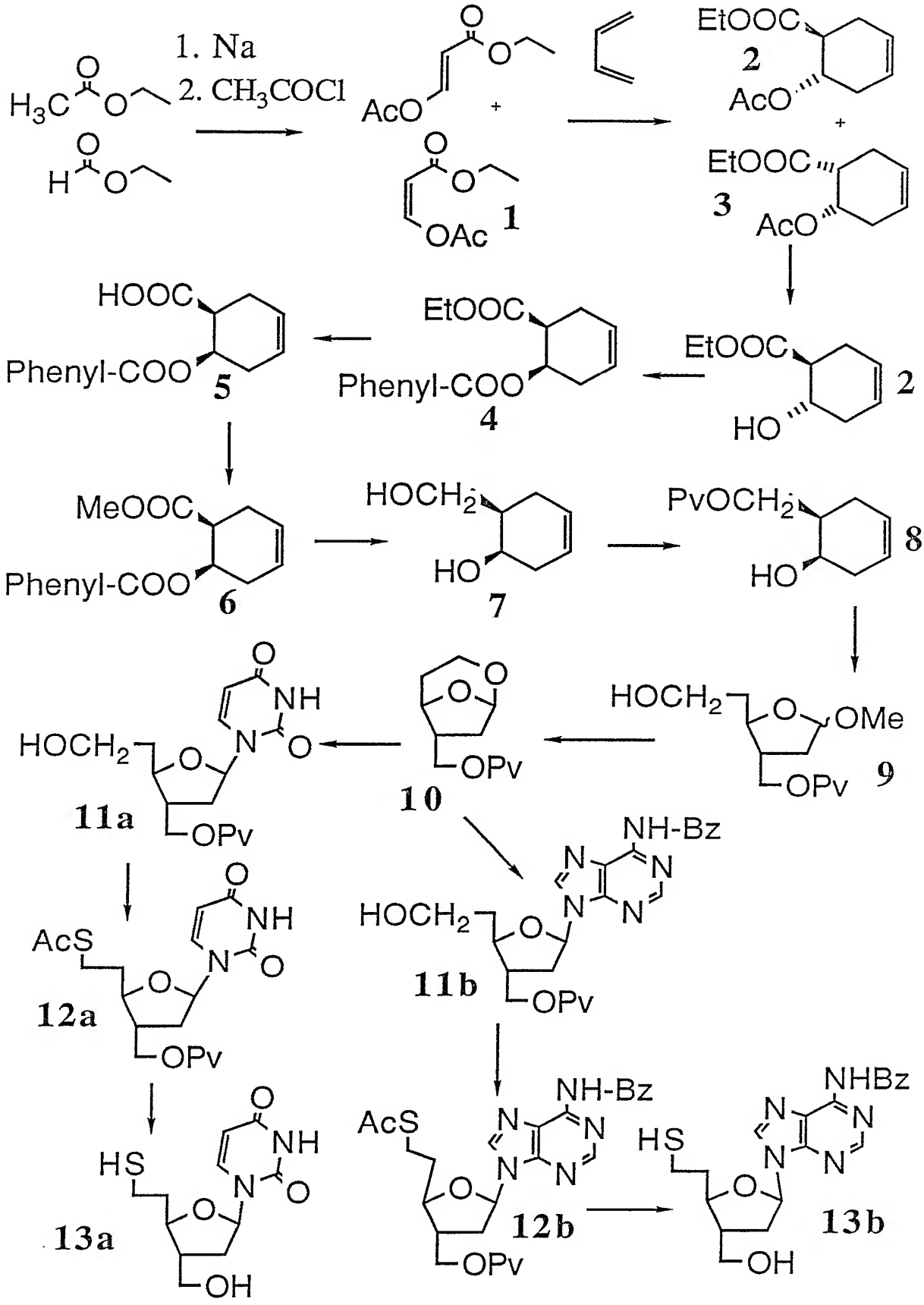


Figure 8 EXAMPLE 3

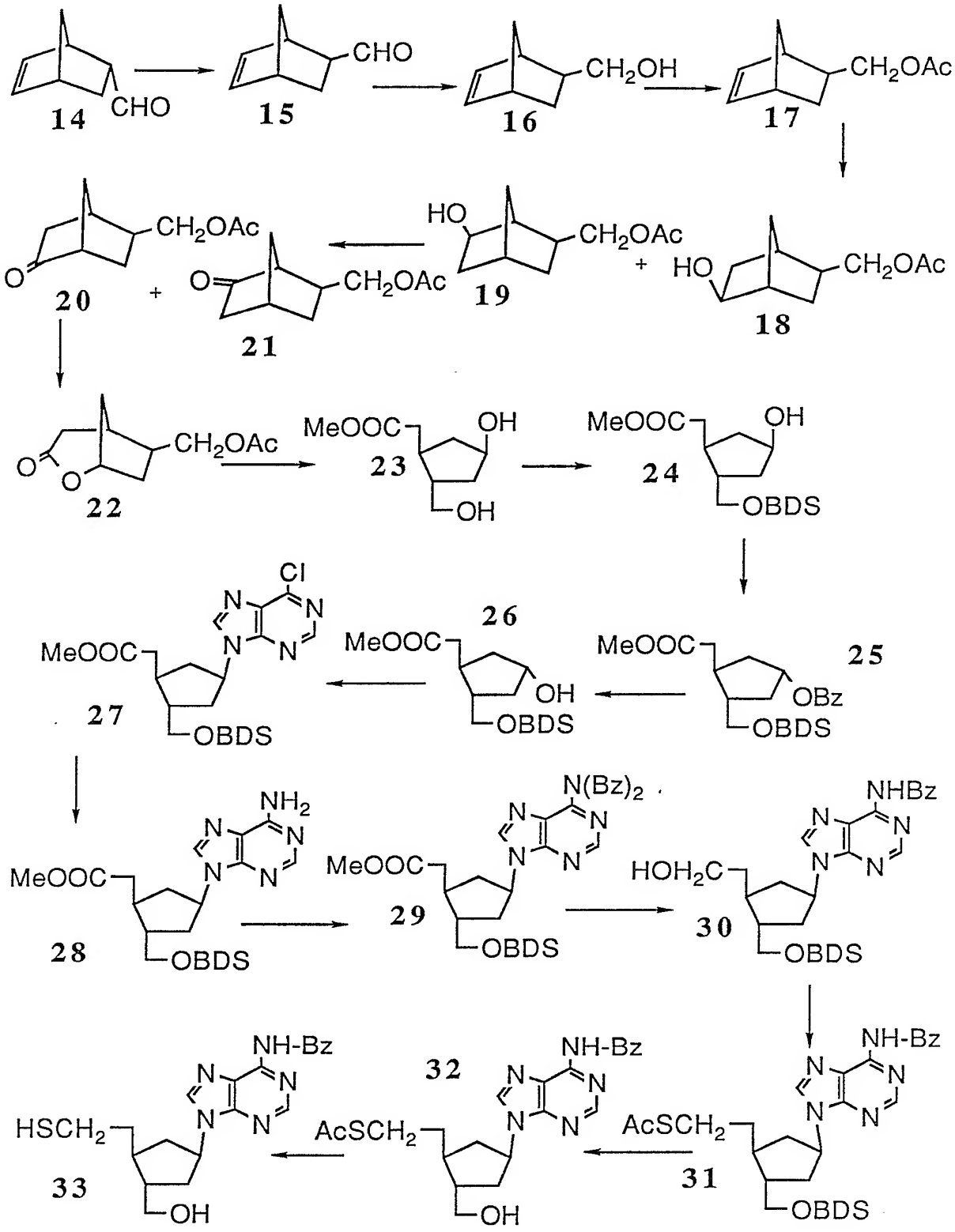


Figure 9 EXAMPLE 4

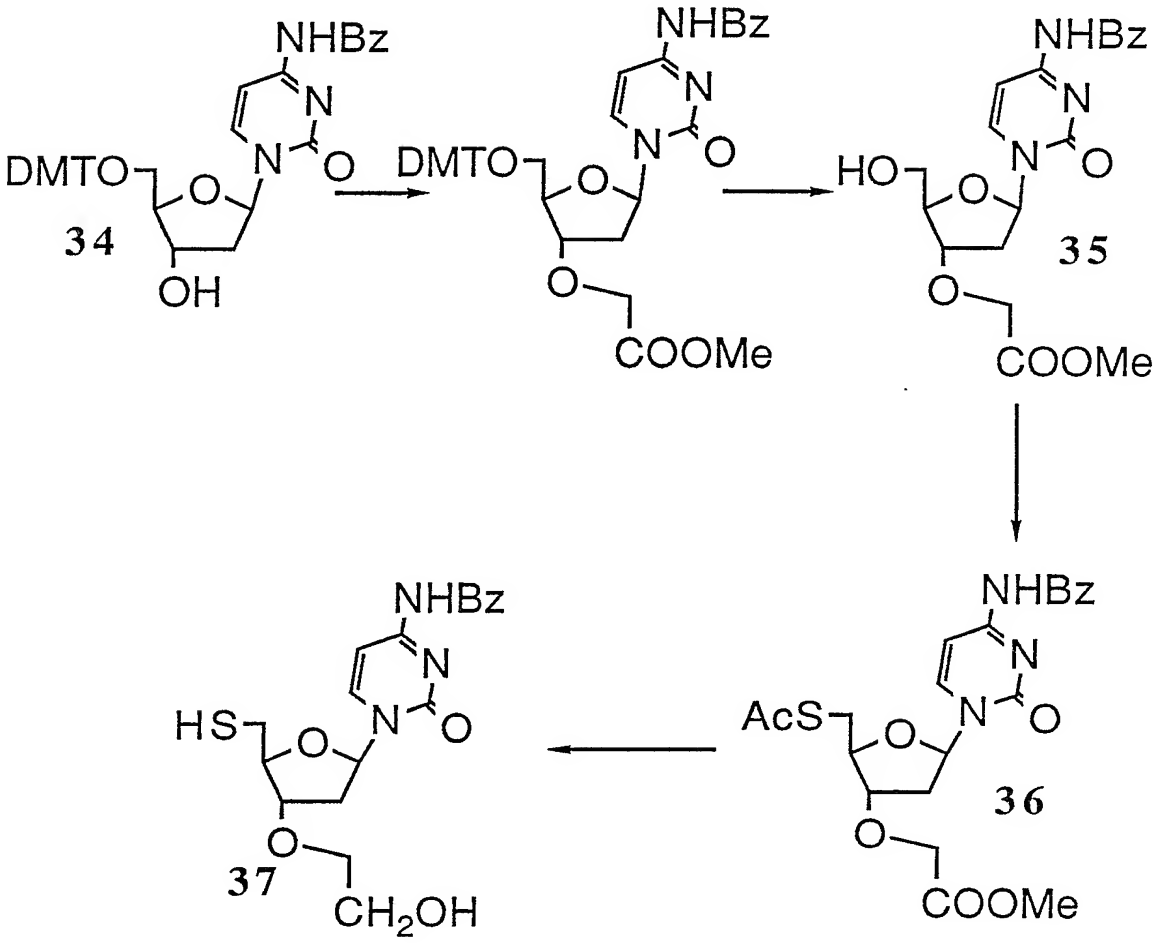


Figure 10 EXAMPLES 5 and 6

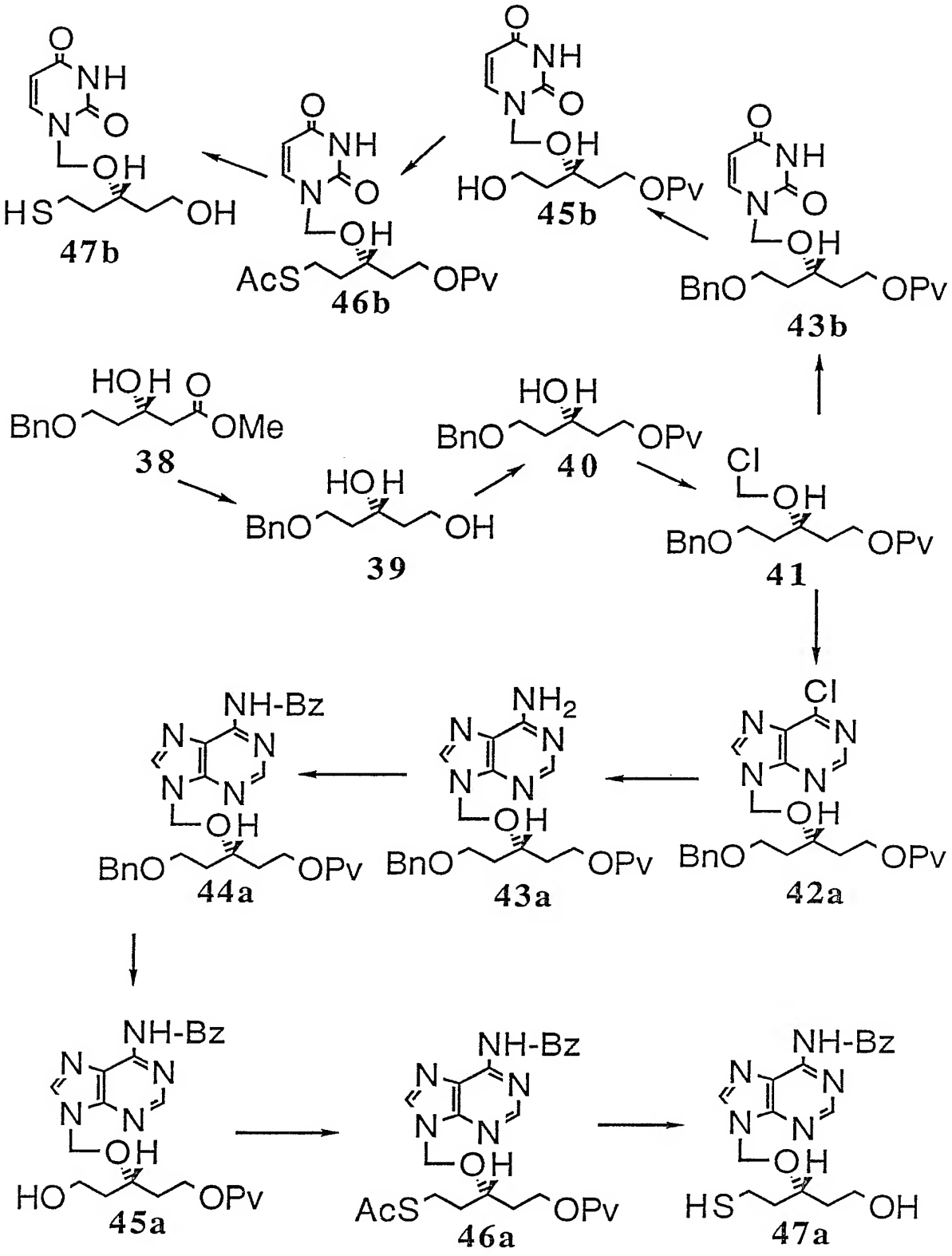


Figure 11 EXAMPLE 7

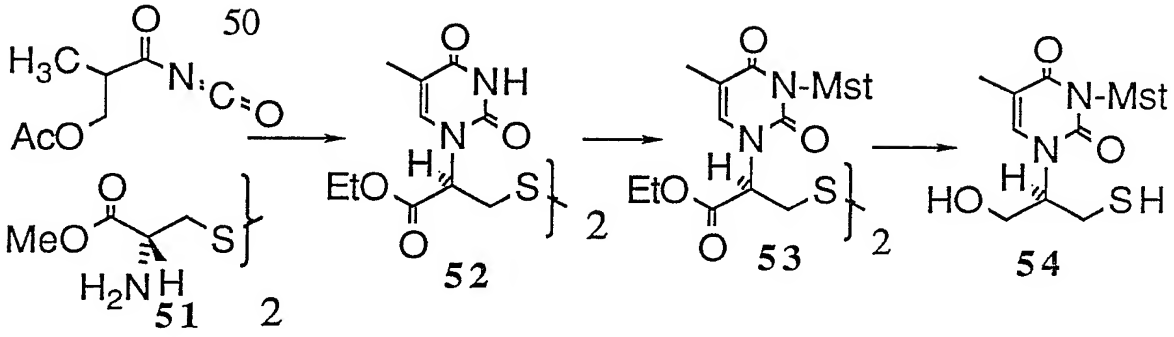


Figure 12
EXAMPLE 8

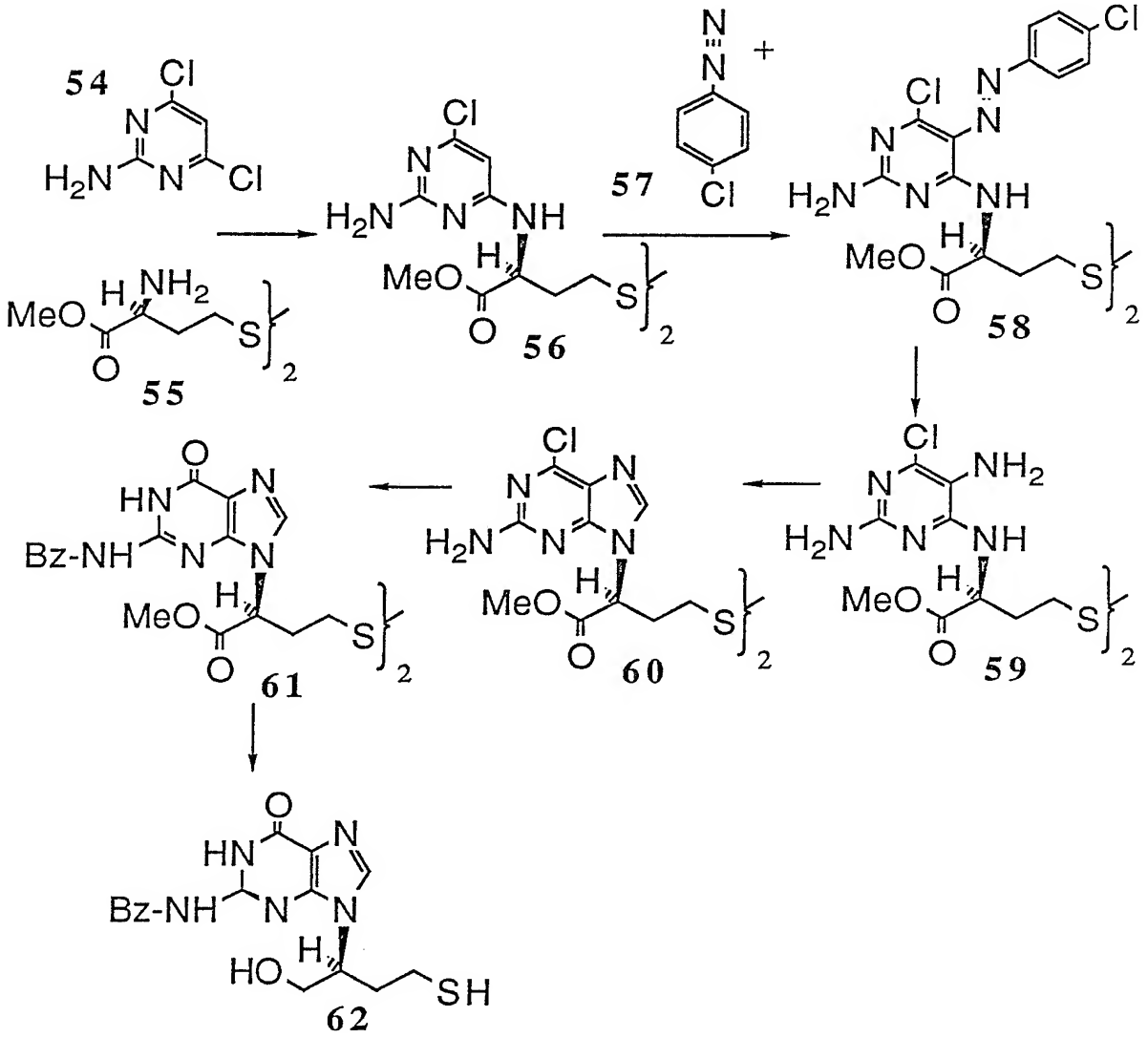


Figure 13
EXAMPLE 9

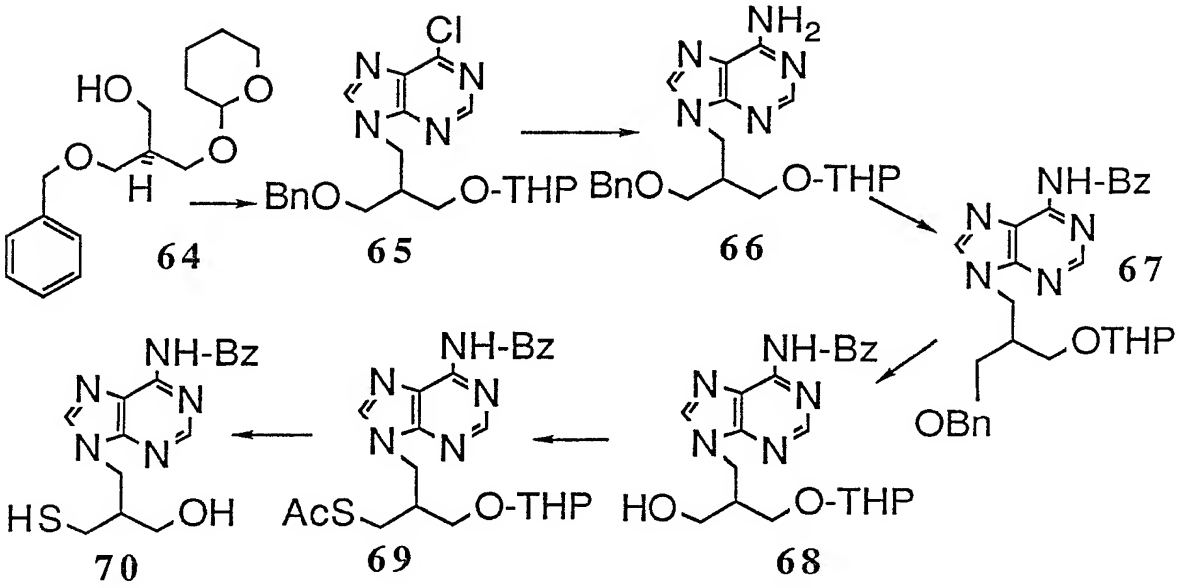


Figure 14
EXAMPLE 10

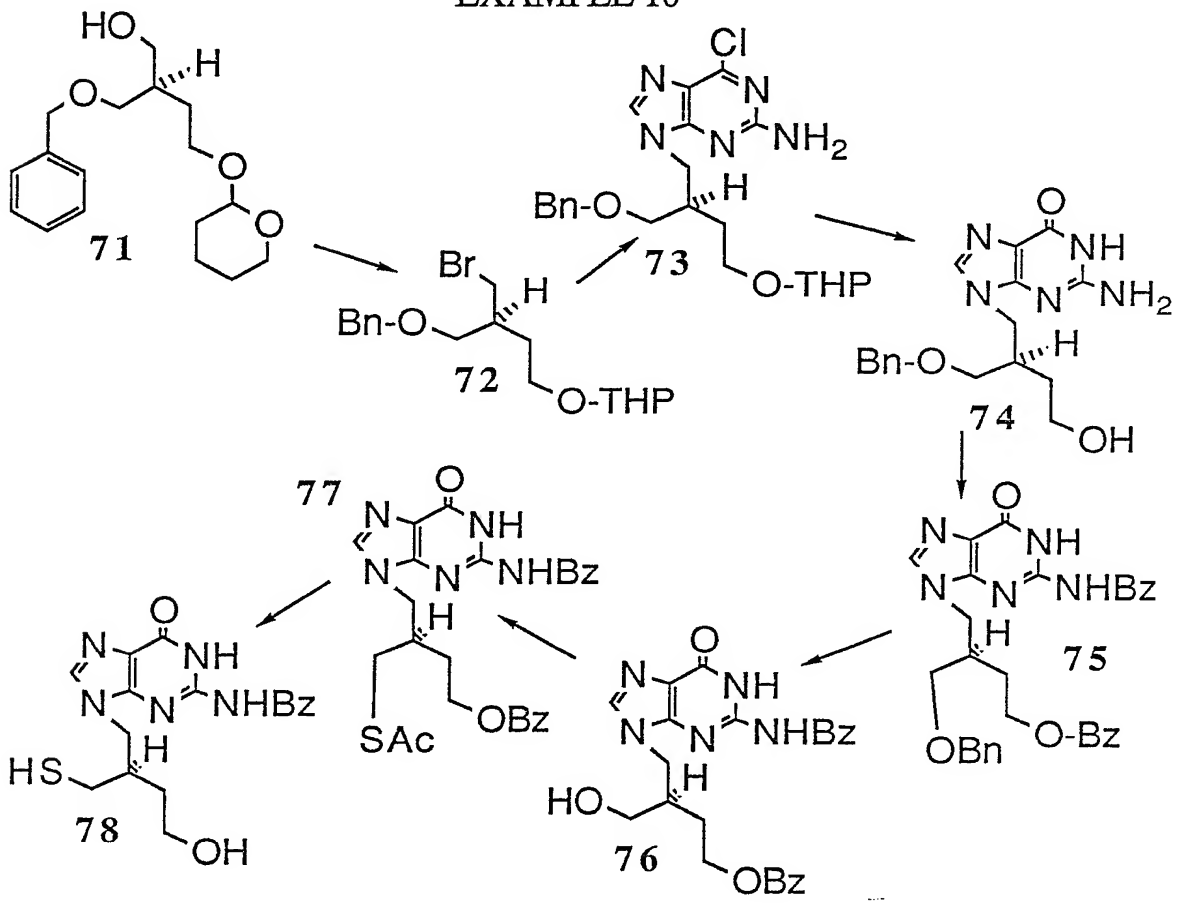


Figure 15

EXAMPLE 11

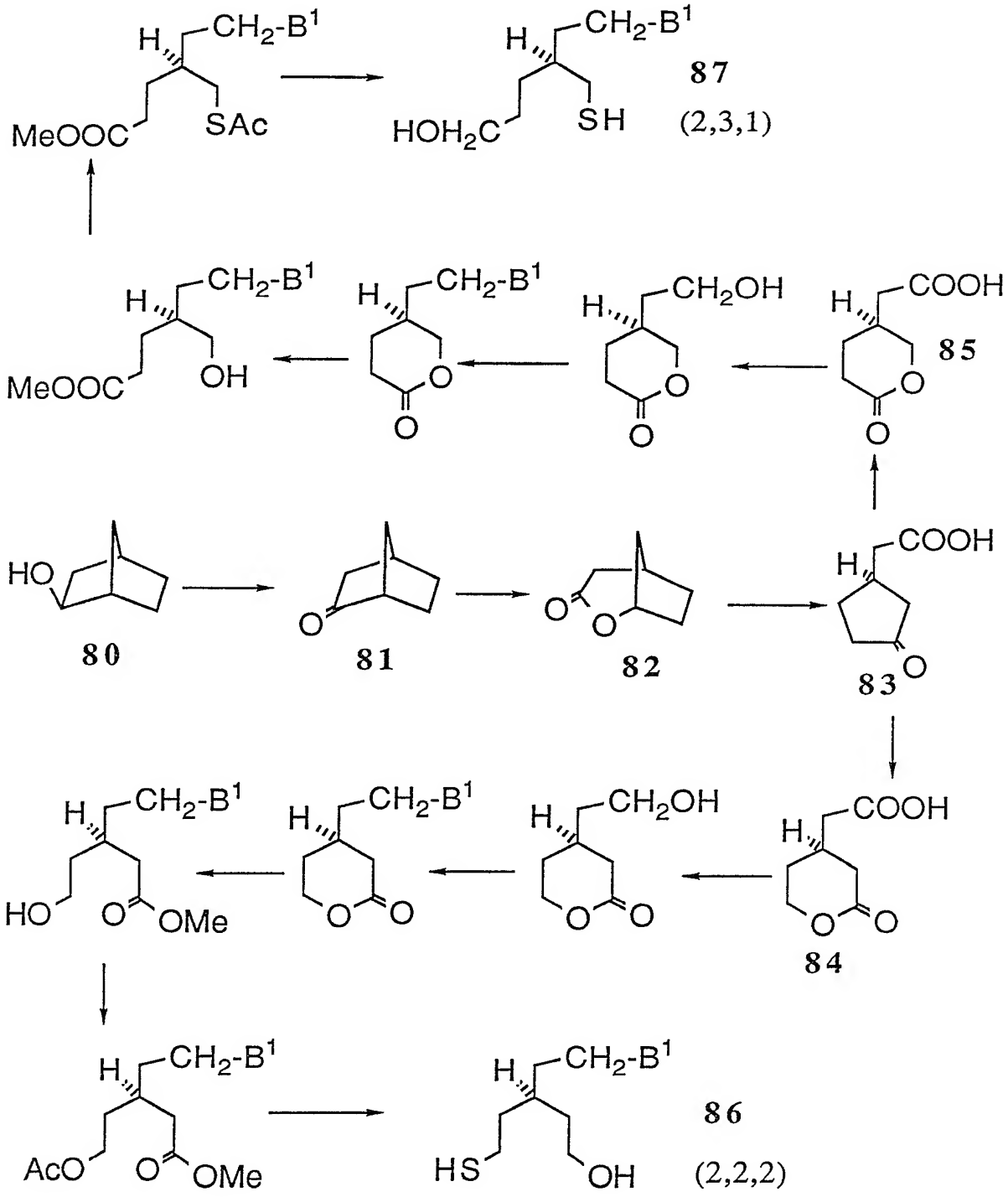
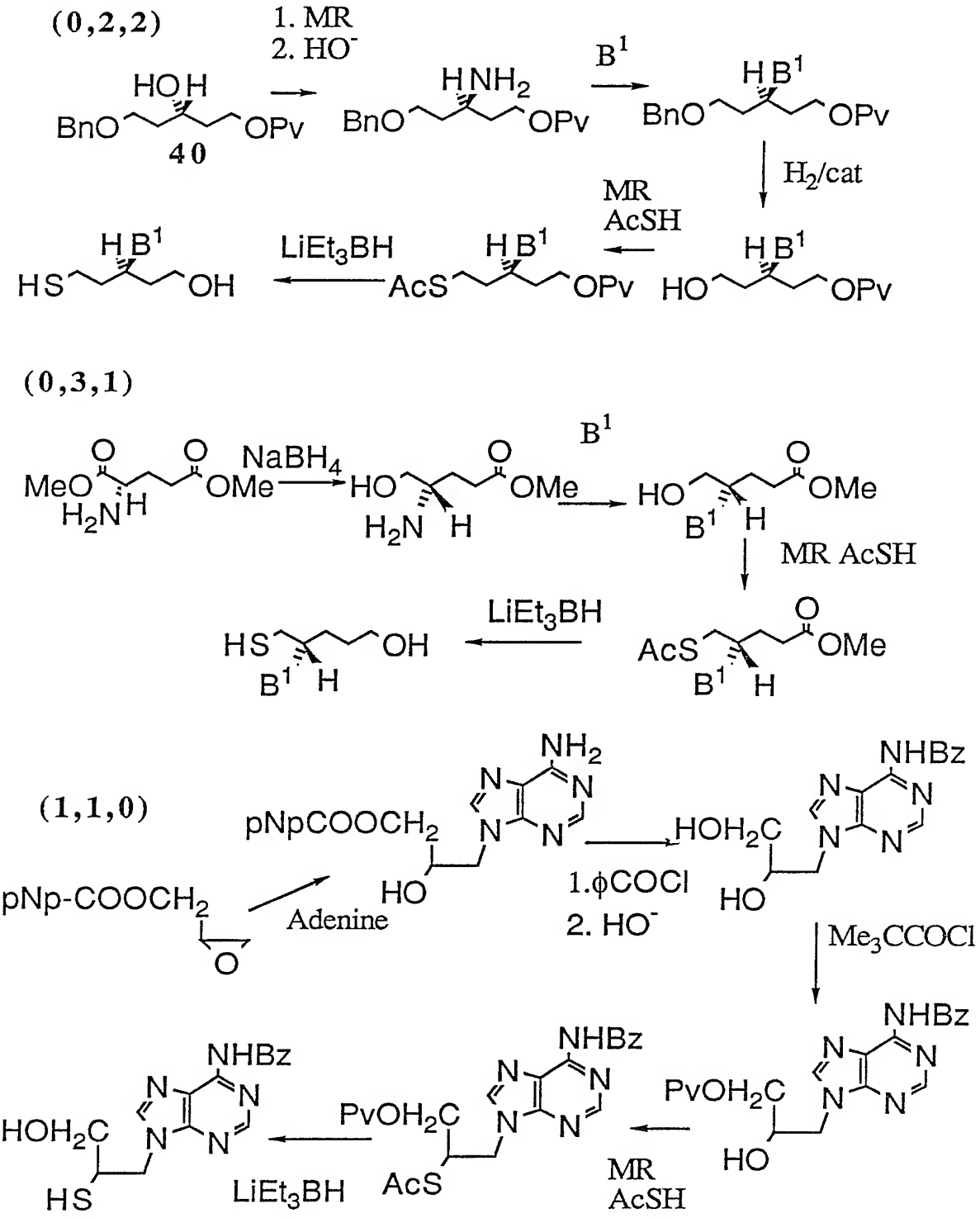


Figure 16

ROUTES TO OTHER FLEXIBLE BUILDING BLOCKS



pNp=p-nitrophenyl MR=Mitsunobu Reaction

Figure 16b

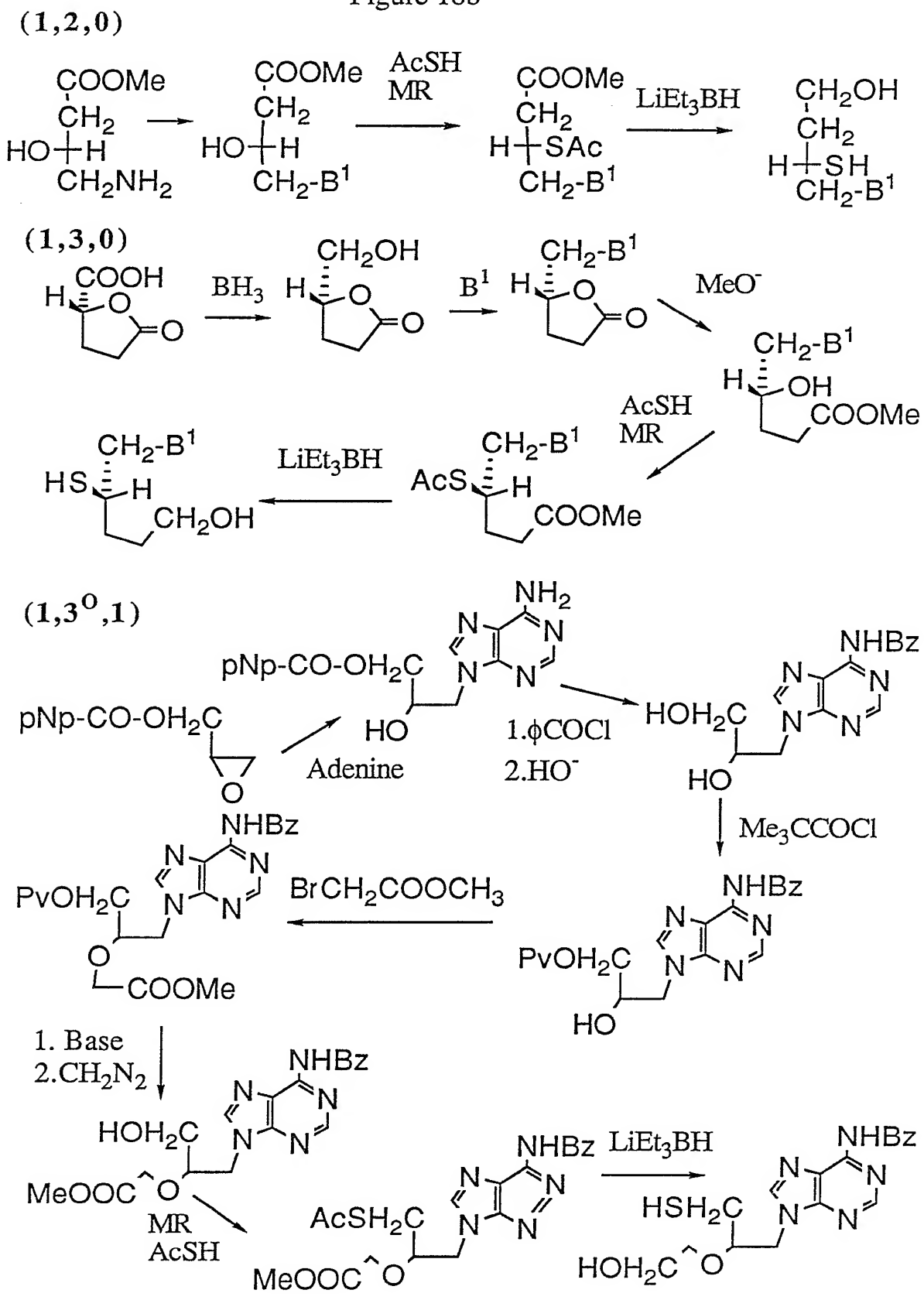
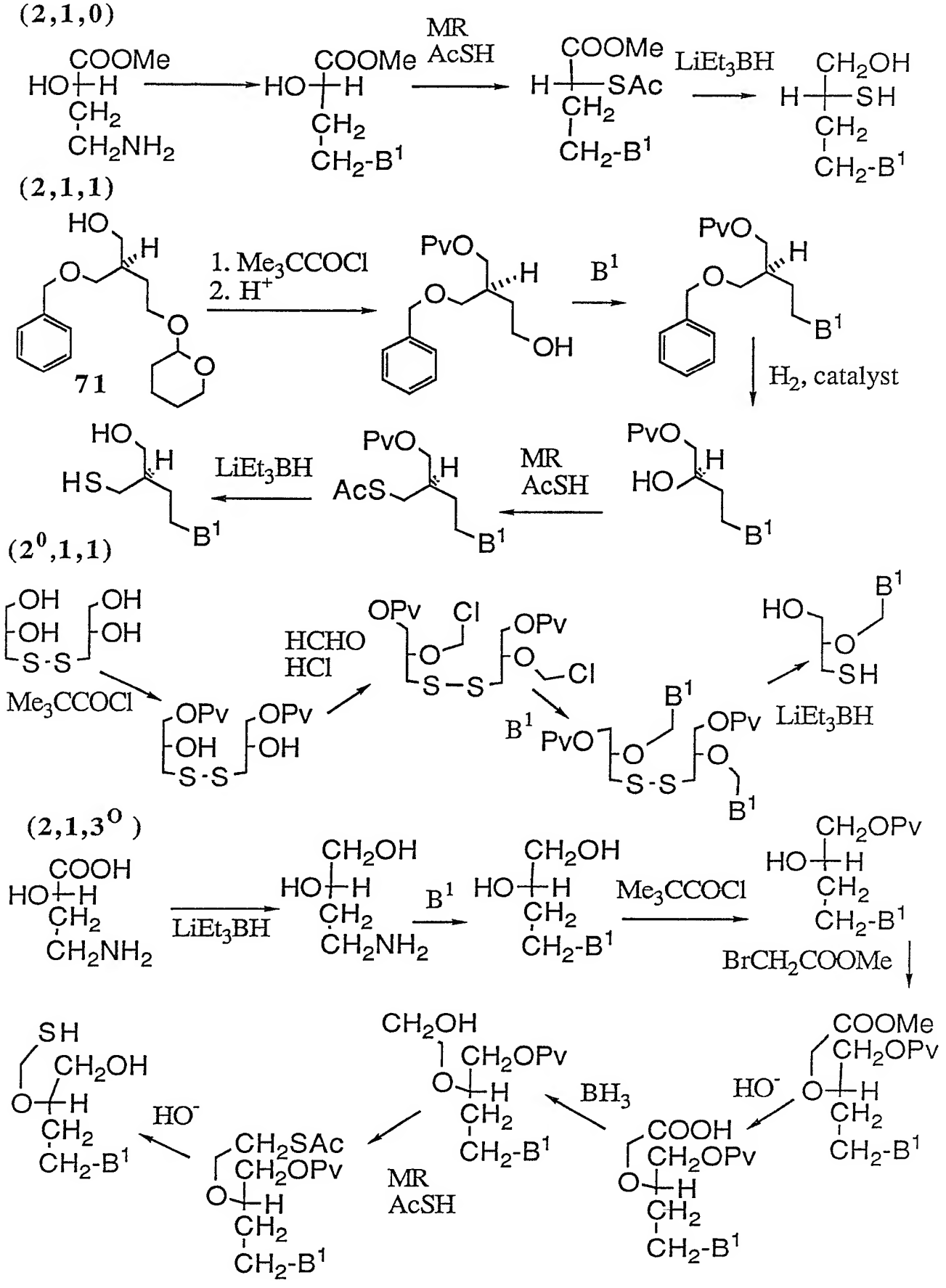

(1,2,0)
COOMe
CH2
HO—H
CH2NH2
AcSH
MR
CH2-B1
LiEt3BH
CH2OH
SAc
SH
(1,3,0)
COOH
BH3
B1
MeO-
OH
COOMe
AcS
HS
(1,3O,1)
NH2
pNp-CO-OH2C
HO
Adenine
1.φCOCl
2.HO-
NHBz
HOH2C
Me3CCOCl
PvOH2C
BrCH2COOCH3
1. Base
2.CH2N2
MeOOC
MR
AcSH
AcSH2C
HSH2C
HOH2C

Figure 16c


(2,1,0)
COOMe
HO—H
CH2
CH2NH2
COOMe
HO—H
CH2
CH2-B1
MR
AcSH
COOMe
H—SAc
CH2
CH2-B1
LiEt3BH
CH2OH
H—SH
CH2
CH2-B1
(2,1,1)
HO
H
O
O
O
71
1. Me3CCOCl
2. H+
PvO
H
O
OH
B1
PvO
H
O
B1
H2, catalyst
HO
H
HS
B1
LiEt3BH
PvO
H
AcS
B1
MR
AcSH
PvO
H
HO
B1
(2^0,1,1)
OH
OH
OH
OH
S-S
Me3CCOCl
OPv
OPv
OH
OH
S-S
HCHO
HCl
OPv
Cl
O
S—S
OPv
O
Cl
B1
B1
PvO
O
S-S
OPv
O
B1
B1
HO
O
SH
LiEt3BH
(2,1,3^O)
COOH
HO—H
CH2
CH2NH2
LiEt3BH
CH2OH
HO—H
CH2
CH2NH2
B1
CH2OH
HO—H
CH2
CH2-B1
Me3CCOCl
CH2OPv
HO—H
CH2
CH2-B1
BrCH2COOMe
SH
CH2OH
O—H
CH2
CH2-B1
HO-
CH2SAc
CH2OPv
O—H
CH2
CH2-B1
MR
AcSH
CH2OH
CH2OPv
O—H
CH2
CH2-B1
BH3
COOH
CH2OPv
O—H
CH2
CH2-B1
HO-
COOMe
CH2OPv
O—H
CH2
CH2-B1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/02323

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): C07F 19/02, 19/04, 21/00
U.S. Cl.: 536/23

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	536/23,27,28,29

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	GB, A, 2,122,198 (ASTRA LAKEMEDEL) 11 January 1984 (11.01.84) See page 12, Example 2.	1-16
Y	Tetrahedron Letters, Volume 22, No. 14, issued 1981 (Great Britain), B. M Trost and D. P. Curran, "Chemoselective Oxidation of Sulfides to Sulfones," pages 1287 to 1290, see complete article.	1-16
Y	US, A, 4,469,863 (TSO ET AL) 4 September 1984 (04.09.84), see "Discussion" in columns 26-29.	1-16
Y,L	Abstract No. 318. North American Meeting of the American Chemical Society, Montreal, Canada, issued 1988, S. H. Kawai, "Single-stranded DNA and RNA binding: Backbone-modified polynucleotide analogues."	1-16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 July 1989	08 SEP 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Gary L. Kunz

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Journal of Medicinal Chemistry, Volume 20, No. 3, issued 1977 (Washington, D.C.) Noronha-Blob et al, "Uptake and Fate of Water-Soluble, Nondegradable Polymers with Antiviral Activity in Cells and Animals," pages 356-359, see Introduction and Discussion.	1-16
A	Journal of Organic Chemistry, Volume 42, No. 4, issued 1977 (Washington, D.C.) Mungall et al., "Carbamate Analogues of Oligonucleotides, pages 703-706, see page 703.	1-16
A	Journal of Medicinal Chemistry, Volume 21, No. 10, issued 1978 (Washington, D.C.) DiMenna et al., "Synthesis of Potential Hypolipidemic Agents: Reaction of Substituted Phenyl-2,3-Epoxypropyl Ethers with Adenine, Uracil, and Thymine," pages 1073-1076, see page 1073.	1-16
A	Tetrahedron Letters, Volume 26, No. 35, issued 1985 (Great Britain) Harnden et al. "An Improved Synthesis of the Antiviral Acyclonucleoside 9-(4-hydroxy-3-hydroxymethyl-1-yl)guanine," pages 4265 to 4268, see structures 1-3.	1-16
A	Biochemistry, Volume 10, NO. 25, issued December 7, 1971, (Washington, D.C.) Pitha et al., "Vinyl Analogs of Polynucleotides," pages 4595 to 4602, see abstract and introduction on page 4594.	1-16
A	GB, A, 2,122,197 (ASTRA LAKEMEDEL) 11 January 1984 (11.01.84), see Example 2 on page 10.	1-16
A	US, A, 4,415,732 (CARUTHERS) 15 November 1983 (15.11.83), see columns 1-2.	17-23
A	US A, 3,846,402 (ECKSTEIN) 5 November 1974 (05.11.74), see entire document.	1-16
T	US, A, 4,808,708 (YOSIDA) 28 February 1989 (25.02.89), see columns 1-2.	1-16
Y	Nucleic Acids Research, Volume 15, No. 7, issued 1987, Seela et al., "Oligodeoxyribonucleotides containing 1,3-propanediol nucleoside substitute," pages 3113-3129, see page 3113, last paragraph.	1-16